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THE PLASMA-COAGULATING PROPERTIES OF STAPHYLOCOCCI

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Submitted for publication August 13, 1936

Cultures of staphylococcus aureus possess a property through which plasma (or blood) may be coagulated. The observations of others, as well as the results to be reported in this article, demonstrate that the coagulating capacity is dependent on a special property of staphylococci through which the fibrinogen is transformed into fibrin clot. This report deals with the results obtained from a study of the action of 34 strains of staphylococci in coagulating plasma and fibrinogen.

LITERATURE

The phenomenon of clotting of blood by staphylococci or products of staphylococci was first mentioned by Delezenne (1) in 1898—though at the time he was studying the *anti-coagulating* properties of certain agents. He observed that small amounts of staphylococcus toxins, B. pyocyaneus toxins, snake venom or peptone, when injected intravenously into dogs, cause a drop in blood pressure and in leucocytes, and prevent the coagulation of the blood. In addition, however, he reported that if the dose that was given was *too* large the blood is either rendered more easily coagulable or the animal is killed by "intravascular clotting."

The first studies comparable to those reported in this paper were made by Loeb (2) in 1903. He found that if a few drops of a broth culture of certain bacteria were added to 3 cc. of blood plasma of the goose, coagulation resulted. Staphylococcus aureus was the most potent of these, and he obtained varying results with pyocyaneus, prodigiosus and coli.

Much (3), in 1908, described the phenomenon in detail. He found that certain strains of staphylococcus aureus coagulated plasma and also coagulated slowly certain exudates containing fibrinogen. "Staphylokinase" was the name given to the active substance. Fibrinogen was not clotted by staphylococci.

Kleinschmidt (4) confirmed Much's findings and made the additional observation that some strains of staphylococcus albus likewise produced clots, though much more slowly. The fibrin formation was only brought about by *living* organisms.

v. Gonzenbach and Uemura (5), in 1916, confirmed the results of Much and of Kleinschmidt, but in addition they reported that plasma may be clotted by heat-killed staphylococci, though not as quickly as by the live organisms. When the cultures were heated to 90°C. for one and one-half hours, their coagulating power was entirely destroyed. They used plasmas from the goat, sheep, man and rabbit and found the last the easiest to clot. Pure *fibrinogen* was not coagulated by staphylococci.

Gratia (6a, b, and d), in 1919, followed closely the changes during the coagulation of plasma by staphylococci, finding that in the process fibrinogen is totally eliminated from the solution. He referred to the "Staphylocoagulase" as a substance capable of inducing coagulation without the mediation of "cytozyme" and "proserozyme" (the "mother substances of thrombin"), since the latter materials were found intact in the residual plasma after coagulation by staphylococci. These studies led Gratia to believe that the activity of staphylococci was similar in some respects to that of thrombin, for they clot the fibrinogen directly, after the platelets and prothrombin are removed. He likewise (6d) was able to obtain the active "staphylocoagulase" in a bacteria-free solution by passing a bacteriophaged culture of staphylococcus through a filter.

Several authors have maintained that the ability of a staphylococcus to clot plasma is a good measure of its pathogenicity. This was noted by v. Daranyi (7) in 1926 and also stressed by Gross (8) in 1927. In addition, Gross concluded from his studies of the staphylococcus and other bacteria (streptococcus, gonococcus, bacillus melitensis, meningococcus, typhoid and dysentery bacilli) that the plasma-coagulation reaction is limited to staphylococcus aureus and is found only in the pathogenic strains. A few strains of pathogenic staphylococcus albus also caused clotting after 24 hours.

Kemkes (9) (1928) likewise emphasized the value of this reaction as an index of the staphylococcus' virulence. He found that all strains from pathogenic sources clotted plasma, whereas the majority of staphylococci encountered as contaminants, secondary invaders, etc., did not. He injected various strains into the kneejoint of rabbits and obtained a striking parallelism in their pathogenicity as determined by this test, their hemolysin production and their plasma-coagulating power. He found also that the "plasma-koagulase" of the staphylococcus could not be inhibited by human serum, horse serum or serum from patients with staphylococcus infection, although the sera contained staphylococcus antihemolysin.

Gross (10) (1928) obtained the coagulating "enzyme" free from the bacteria themselves in the Seitz filtrate of broth cultures 3 to 14 days old, and with it clotted citrated rabbit plasma. The tests which he considered the most valuable for determining a staphylococcus' pathogenicity were the plasma-coagulating reaction, the hemolysin production, gelatin-liquefaction, and the lesions produced in rabbits following intracutaneous injections. The same author (11), in 1931, found that the potency of filtrates of staphylococcus cultures paralleled the activity of the cultures themselves. The coagulating substance resisted heating

at 90°C. for one hour. By injecting rabbits intravenously repeatedly with small amounts of active culture filtrate, he was unable to produce in the animals antibodies that had the ability to prevent staphylococcal coagulation.

In a later article (12) the same author compared the plasma-clotting substance of staphylococci with other substances found in the same cultures and filtrates, *i.e.*, the leucocidins, the hemolysins, the skin-necrotizing and animal-killing substances. The last two were considered to be a single entity. Qualitative differences existed, however, between the toxic properties and the plasma-clotting substance in the staphylococcus filtrate: the former are heat labile, while the latter is quite heat stable; the coagulating property appears in the culture at a much earlier period in its growth, and immune bodies are formed to the toxic substances but not to the coagulase.

Authors differed in their views as to whether or not *fibrinogen*, chemically derived from plasma, could be clotted by staphylococci. Gratia (6e) (1920) found that staphylococci could clot fibrinogen if he added to the latter a solution of plasma from which fibrinogen and the elements of thrombin had been extracted. Gengou (13) (1933) pointed out that the reason the fibrinogen is coagulated under such circumstances is most likely because some substance in the added solution aids coagulation, whereas Gratia's theory was that the staphylococci were given in this way a better medium in which to grow and so formed a more potent coagulase. Gengou prepared "staphylocoagulase" free from the organisms themselves by growing the staphylococci in the serum from a plasma-calcium clot, centrifuging the bacteria out and then heating the supernatant to 60°C. This solution coagulated plasma and fibrinogen. He used that method of preparation of bacteria-free coagulase because he found that Seitz and Chamberland filtrates of broth cultures were inactive.

In commenting on the possible importance of the coagulating properties of staphylococci in human infections, Gross (14) states that it is well recognized that in operations on staphylococcus infections, such as carbuncles or osteomyelitis, the blood clots quickly, whereas in others, notably streptococcus infections, continued bleeding is one of the most feared complications. In these cases he feels that the staphylococci may play a rôle in making the blood more coagulable. It is likely also that in staphylococcus infections antibodies are formed to the coagulating principle, though this likewise is mostly theoretical. If such antibodies are present, they may play a part in preventing more frequent thromboses in infections with staphylococci. Sudhues and Schimrigk (15) (1933) found that normal plasmas and those from patients ill with staphylococcus infections were clotted equally well and quickly by staphylococci, even though the staphylococcus anti-hemolysin reaction indicated that in some of the cases antibodies were present in the blood. The plasma of rabbits sensitized to the staphylococcus showed no clot-inhibiting antibody, although Gross (above) had referred to a clot-inhibiting action of "antitoxic staphylococcus sera."

Chapman and coworkers (16) (1934) regarded the "coagulase test" as an index of the pathogenic properties of the staphylococcus. The virulence was determined

by the ability of the strain to kill a rabbit within 10 days after intravenous inoculation. They recommend as tests for pathogenicity of staphylococci the simultaneous determination of their hemolytic and coagulating activity, and a careful study of pigment production on solid media after 48 hours' growth.

Gengou (20) found that "staphylocoagulase" can bring about agglutination of platelets in test tubes, and that "staphylotoxine" may change these agglutinated platelets into a homogeneous viscous mass. He pointed out that those are the changes that take place in the formation of a thrombus, and indicated that these properties of staphylococci offer a possible explanation for the occurrence of "spontaneous" postoperative thrombi.

Walston (21) obtained the coagulating substance by precipitation with alcohol, acetic acid or ammonium sulphate. After alcoholic precipitation this principle was dialysable through cellophane, whereas before this step it was not. Some human sera, from normal individuals, were found to inhibit the plasma-coagulating reaction. So far, he has not been successful in attempts to produce resistance to clotting in the plasma of rabbits injected with the coagulating substance.

A summary of the reports of various authors, therefore, on the subject of staphylococcus clotting of plasma and fibrinogen, indicates that this property occurs most frequently in strains of the aureus group, and is most uniformly found in cultures of virulent strains. According to some reports, it may at times be obtained free from the cell bodies of the bacteria. The coagulating substance appears in broth cultures within the first three days of incubation. It can best be obtained in filtrates of cultures about 3 to 6 days old and is effective with plasmas of different animals but at different rates of speed. It is like thrombin and yet it appears to differ from it in at least one respect: it does not neutralize hirudin, so-called antithrombin. Most workers have found it to be very heat stable. It appears from the studies of several authors that the plasma-coagulating reaction serves as a good test of the organisms' pathogenicity, as there is a definite parallelism between their virulence as shown by various tests and their ability to clot plasma. Several workers have found that pure fibrinogen may likewise be clotted by the staphylococcus or by staphylococcus cultures freed from the organisms through filtration or centrifugalization, though this reaction is not nearly as constant nor as easily obtained as that with plasma.

The following points were the chief ones investigated in the present study: number of strains active in clotting plasma, with the degree and speed of activity; the cultural conditions; characteristics of the active substance; different conditions applicable to clotting of fibrinogen in contrast to plasma; the results of injections into animals.

MATERIALS AND METHODS

Plasma. Normal human and rabbit plasmas were employed for the tests. These were prepared by taking the blood under sterile conditions into dry potas-

sium oxalate in such amounts as to yield a final dilution of oxalate in blood of 0.2 per cent for human and 0.4 per cent for rabbit plasma. Citrate may be used as an anticoagulant equally successfully. The oxalated or citrated blood is then centrifuged, and the plasma pipetted off. After numerous experiments it was found that diluted plasma was more satisfactory for the tests than undiluted material. A 1:5 dilution of plasma was, therefore, regularly employed in the observations reported in this article. Plasma, if kept sterile and in the icebox, may be used for 2 to 3 weeks.

Fibrinogen. Fibrinogen was precipitated from plasma (human or rabbit) by two methods: one-half saturation with sodium chloride and 25 per cent saturation with ammonium sulphate. Both methods were satisfactory, but the ammonium sulphate yielded more precipitate and was used for most of the work. After the ammonium sulphate or the sodium chloride had been added to the plasma, the precipitate of fibrinogen was centrifuged out and then redissolved in an amount of normal saline solution equal to the original amount of plasma used. This process was repeated again in order to obtain a purer solution of fibrinogen.

Cultures. Plain meat infusion broth was regularly employed for culture medium. The cultures were incubated for periods ranging from 16 hours to 2 weeks. A light or medium growth is more suitable than a very heavy one. An equally satisfactory method (in some strains at least) consisted in removing staphylococci from the culture medium and resuspending them in an amount of salt solution sufficient to produce a density approximately equal to that of a 24 to 48 hour culture. Tubes of diluted plasma and fibrinogen alone served as controls of the absence of spontaneous coagulation.

Sterility was strictly adhered to throughout the experiments. Its importance will be emphasized by results which will be reported in the latter part of this article and which were obtained with certain contaminating bacterial species encountered during the investigation.

Hemolysin production by the strains of staphylococci was estimated by subcultures on the surface of blood agar.

Pigment production was estimated by the color produced by subcultures in plain agar and Loeffler's medium.

DURATION OF CULTURAL ACTIVITY AND INFLUENCE OF pH

The optimal period of growth of the broth cultures was from 16 hours to 1 week for the production of an active culture (or filtrate). Some cultures will remain active after standing at room temperature for three months, but the majority of the strains are inactive by that time, many losing their clotting power within one month. It is necessary for the pH of the cultures to be within certain limits for satisfactory determinations of their clotting power. The optimal range of pH is about 6.2 to 7.4, but cultures within the limits of 6.0

and 8.4 can clot plasma, though the reaction is a slow and incomplete one when the pH is at the outer limits of these values. When cultures stand for some time at room temperature or in the incubator, the pH becomes very high (8.4 or over), due presumably to the dissolving of alkali from the flask. If grown in dextrose broth, the pH goes down below 5.2 and they become completely inactive—even inhibiting clot formation when calcium is added to a plasma-staphylococcus mixture.

Filtrates. Broth cultures were filtered through Berkefeld and Seitz filters and then tested with plasma and fibrinogen in the same way as were the original cultures. The Berkefeld W and Seitz filters were found to retain most of the active substance and so were not used for the routine tests. The results recorded deal with filtrates from the V and N Berkefeld candles.

During the course of the filtration experiments it was learned that calcium may be washed through the Berkefeld and Seitz filters in concentrations high enough to clot plasma, and thus may be the cause of some confusing results. Both the Berkefeld and Seitz filters are known to contain a considerable amount of calcium and the following experiments proved that the calcium may be washed off them in quantities sufficient to clot plasma. If a few cubic centimeters (10 to 20 cc.) of normal saline solution or of broth are passed through either of the filters, the filtrates form clots when added to plasma. When, however, the filter is first washed through with 50 to 100 cc. of saline and then more filtrate is collected at the end of this filtration, it no longer contains the coagulating substance. The following tests were done which identified this plasma-clotting substance as calcium. The first few cubic centimeters of saline or broth filtrate which could clot plasma were tested with a weak solution of potassium oxalate (2 to 4 drops of this to 0.5 cc. of the filtrate). The result was a definite white precipitate and the fluid no longer was able to clot plasma. Saline solutions or broth before filtration gave no precipitate when tested in this way, and the filtrates which had no activity with plasma either showed only a very light precipitate or none at all. Through the kindness of Dr. Harold Blumberg of the School of Hygiene of the Johns Hopkins University, spectrographic studies were made of the material which washed through the filters.

He found a definite, strong band in the spectrum characteristic of calcium. No bands indicating magnesium or barium were detected. Other tests corroborated the spectrographic observations.

The following routine procedure was adopted for the experiments with filtrates: 50 to 100 cc. of sterile normal saline were first passed through the filter and discarded; a few more cubic centimeters were then filtered through, transferred to a sterile tube and tested for their plasma-coagulating activity. The staphylococcus culture being investigated was then filtered through the same candle and tested with plasma or fibrinogen, but the results were recorded only when the saline filtrate preceding it had shown a negative reaction with plasma. These tests demonstrate the way in which a small amount of calcium in fluids may confuse the results when studying their plasma-clotting activity, and emphasize the importance of having a control filtrate of an inactive fluid such as saline.

Technique of test. After numerous trials it was found that the proportions of the various materials used in the tests were important in order to obtain the most uniform and accurate results. Consequently, quantities of the various reagents, which experience indicated as most satisfactory, were regularly employed. The combinations of materials were as follows:

1. *Broth cultures* plus *plasma.* 0.5 cc. broth culture plus 0.5 cc. of a 1:5 dilution of plasma.

2. *Broth cultures* plus *fibrinogen.* 0.2 cc. of diluted broth culture plus 0.5 cc. of a 1:5 dilution of fibrinogen.

3. *Agar cultures* plus *plasma.* A platinum loopful of organisms plus 0.5 cc. of a 1:5 dilution of plasma.

4. *Sterile filtrate* plus *plasma.* 0.5 cc. filtrate, plus 0.5 cc. of a 1:5 dilution of plasma.

5. *Sterile filtrate* plus *fibrinogen.* 0.5 cc. filtrate, plus 0.5 cc. of a 1:5 dilution of fibrinogen.

The contents of all tubes were thoroughly mixed.

Tests were carried out at room temperature and in the water bath at 37°C. Since no difference, either in the speed or completeness of the reaction, was noted at the two temperatures, the tubes were usually left on the laboratory desk. Coagulation likewise took place, though not quite as quickly when the tubes were put into the icebox immediately after adding the staphylococci to the plasma, thus showing that growth of the organisms in the plasma was not necessary for the production of clotting.

The end point of the reaction was determined by slowly inverting the tubes and noting the formation of fibrin. The degree of coagulation was recorded by +

TABLE I

Clot formation by active and inactive strains

STRAIN	SOURCE	COLOR (DEGREE OF AUREUS)	HEMOLYSIS	PLASMA COAGULATION-TIME			
				Human plasma		Rabbit plasma	
				Culture	Filtrate	Culture	Filtrate
By active strains							
Pugh	Acute abscesses (Leukemia)	Aureus +++	+++	30 min.*	16 hrs.	30 min.	16 hrs.
201	Foot-pad disease (rabbit's)	Aureus +	+++	10 min.	10 min.	10 min.	10 min.
GVS	Foot-pad disease	Aureus +	0	30 min.	2 hrs.	30 min.	15 hrs.
FST	Stye	Aureus ++++	++	1 hr.	16 hrs.	30 min.	16 hrs.
GFS/S	Sinus	Aureus +	++	16 hrs.	20 hrs.	2 hrs.	20 hrs.
Crom	Lung abscess	Aureus +++	++	30 min.	16 hrs.	30 min.	20 hrs.
VV	Stye	Aureus +++	+++	1 hr.	16 hrs.	30 min.	16 hrs.
FS	Stye	Aureus ++++	++	30 min.	16 hrs.	30 min.	30 min.
Sm	Eye infection	Aureus +++	++	1 hr.	16 hrs.	45 min.	16 hrs.
PR	Osteomyelitis	Aureus +	++	1 hr.	18 hrs.	1 hr.	18 hrs.
Earl	Laboratory contaminant	Aureus ++++	++	2 hrs.	16 hrs.	2 hrs.	16 hrs.
Dun	Bed-sore	Aureus +++	+++	1 hr.	0	1 hr.	0
Rain	Furunculosis; osteomyelitis	Aureus +++	+++	30 min.	0	45 min.	0
Est	Mastoid	Aureus +++	+++	1 hr.	0	1 hr.	
Grif	Knee	Aureus +++	+++	1 hr.	40 hrs.	2 hrs.	20 hrs.
Ha	Blepharitis	Aureus ++	+++	45 min.	0	1½ hrs.	0
Ha/R	Blepharitis	Aureus +++	+++	2 hrs.	0	30 min.	
Dub	Recurrent furuncles	Albus	++	2 hrs.	16 hrs.	2 hrs.	16 hrs.
Tal	Carbuncle	Aureus +++	+++	1½ hrs.	0	1½ hrs.	0
Smith	Septicemia	Aureus ++	+++	30 min.	10 hrs.	30 min.	18 hrs.

HLE	Impetigo	Aureus +++	+++	1 hr.	0	18 hrs.	0
U103D2	Foot-pad disease	Aureus +	+++	45 min.	0	45 min.	0
Just	Chronic skin infection	Aureus +++	+++	1 hr.	0	1 hr.	0
SB	Impetigo	Aureus ++	+++	45 min.	0	18 hrs.	0
Jack	Styes	Aureus ++	++	1 hr.	0	1 hr.	0
	Septicemia	Aureus +++	+++	1½hrs.	0	1½ hrs.	0
Number active				26	14	26	14
Average time				1 hr. (excluding 1, 16 hrs.)	16 hrs.	1 hr. (excluding 2, 18 hrs.)	14 hrs.
Inactive strains							
SS	Vernal catarrh	Aureus +	0	0		0	
201 E/O	Non-hemolytic variant of 201	Aureus +	0	0		0	
WPF	Eczema	Albus	0	0		0	
OG	Vernal catarrh	Albus	+	0		0	
Ha/Rab	Variant of Ha	Albus	0	0		0	
VAM	Epidermophytosis	Albus	+	0		0	
Cap	Eczema	Albus	+	0		0	
AT	Gonorrhea (eye)	Aureus ++	0	0		0	

* The times given are for complete coagulation.

signs. ++++ indicates complete coagulation of the contents of the tube. + indicates the formation of a few strands of fibrin. +++ and ++ are intermediate between the two extremes.

RESULTS OF EXPERIMENTS

1. Degree of activity. Thirty-four strains of staphylococci were tested for their plasma-coagulability and 26 (or 76 per cent) of these were found active. There was a marked variation, however, in the speed of clot formation by the various strains and a slight variation in degree. The majority (19 strains) produced solid clots (++++), and the rest gave clots not quite as solid and designated as +++ (see Table I).

2. Speed of plasma-clot formation. The fastest time for clotting human plasma was 10 minutes and the same for rabbit plasma. The average time of 25 strains for human plasma was one hour. One strain was unusually slow, requiring 16 hours. The average time of 24 strains for rabbit plasma was likewise one hour; two strains induced clot formation in rabbit plasma only after 18 hours.

3. Pigment formation. Twenty of the active strains were definitely aureus and one was albus; the remaining five were borderline in color between aureus and albus, as they showed a very light yellow color only after remaining on the desk for 48 hours on plain agar or on Loeffler's medium. Of the 8 strains which failed to coagulate plasma, one was aureus, two were very light yellow and five were albus. In other words, of the 23 aureus strains, 20 clotted plasma, and of the 6 albus strains 1 clotted plasma.

4. Hemolytic power. Of the active strains 16 produced marked hemolysis around the surface colonies on blood agar plates, 9 showed moderate hemolysis and one showed none. Of the 8 inactive strains, 2 were moderately hemolytic under these conditions, 2 others were slightly so and four were not. Or in other words, all but one of the active strains were hemolytic and 4 of the 8 inactive ones were not hemolytic.

ACTIVITY OF THE FILTRATES

Broth cultures were filtered according to the method described, and it was found that certain strains regularly produced an active filtrate. Of the 26 plasma-coagulating strains studied for their fil-

trate's activity, the filtrates from 14 clotted plasma. The most rapid coagulation for both human and rabbit plasma was 10 minutes, but this was done by only one strain, the rest taking much longer. The average time for the coagulation of human plasma was 16 hours and for rabbit 14 hours (Table I).

The reaction of these filtrates when added to plasma indicates that in the case of the strains whose filtrates produce coagulation the active substance is excreted into the culture media, whereas with the other strains, whose cultures are active but whose filtrates are not, the potent substance is apparently intimately connected with the live staphylococci and does not pass through the filter. It is interesting to note that the discrepancy between the activity of whole culture and its filtrate could not be anticipated. For example, the 12 strains whose filtrates did not clot plasma produced clots just as quickly and as completely as did the other 14.

Filtrates from highly active strains may be diluted to varying degrees with normal saline and still clot plasma. The majority of the more active ones in this series coagulated plasma well at a dilution of 1:20, many at 1:40 and a few produced a slight clot at 1:100.

CHARACTERISTICS OF THE ACTIVE SUBSTANCE

1. Effect of heat. The heat resistance of the coagulating substance varied markedly with the strain of staphylococcus under consideration; some were quite heat labile and a few were extremely heat stable. The heat resistance of 11 strains of staphylococci was studied and the following results were obtained: in 2 the coagulating activity was almost gone after exposure to 60°C. for 5 minutes and completely gone after 30 minutes; in 2 others, inactivation required heating at 60°C. for one hour, and in 3, 80°C. for one hour was necessary. There were 4 strains, however, which remained partially active even after being subjected to 100°C. for one-half hour. Of these 4 heat-stable strains only 2 ("201" and "G.V.S.") retained their coagulating activity toward human plasma, but all of them still clotted rabbit plasma after exposure to this high temperature. The bacteria-free filtrates of the heat stable strains likewise resisted the same high temperatures. In order to determine whether or not the heat resistance of the cultures might vary with their age, cultures of 3 strains representing

different degrees of thermal susceptibility were tested after growth for 24 hours, 3 days and 6 days. Only a slight difference was noted, in that 2 of the 6-day cultures appeared to be slightly more susceptible to heat than their younger cultures.

2. Alcohol precipitation and concentration. The active principle could be precipitated out with 3 or 3½ volumes of 95 per cent alcohol. When the active filtrate was treated in this way, a light precipitate was formed, which was then centrifuged out, allowed to dry, under sterile conditions, and dissolved in normal saline in an amount equal to the original filtrate used. This solution clotted human or rabbit plasmas actively. If the precipitate was allowed to dry overnight and then taken up in saline, the solution was still able to clot plasma, but did so more slowly than if dissolved immediately after precipitation. By dissolving the alcoholic precipitate in an amount of saline equal to half the volume of filtrate, a solution was obtained which equalled in potency the original material. Most of these tests were done with one exceptionally potent culture filtrate ("201"). One drop of either of the two fluids (from strain "201") was enough to clot 0.5 cc. of plasma, though the filtrate produced the clot more quickly (1 to 2 hours) than did the alcohol precipitate (more than 2 hours).

The active material obtained by precipitation with alcohol could be concentrated. For example, a sample of filtrate from the same culture was divided into two parts and each treated with alcohol in order to precipitate out the active substance. The precipitate of one of these was dissolved in an amount of saline equal to the original volume of filtrate and the other in one-tenth as much. The concentrated solution produced a clot more rapidly than the other and was likewise more active than the original filtrate. The heat resistance of the saline solution of the alcoholic precipitate was equal to that of the original filtrate.

Table II illustrates the way in which the coagulating property of 3 representative strains can be concentrated by alcohol precipitation and then dissolving the precipitate in saline in one-tenth or one-twentieth the original amount. When the most concentrated alcohol precipitates are allowed to stand in the icebox a few days some fine crystals separate out, leaving a clear supernatant fluid above. The coagulase and the fibrinolytic substance are both found almost en-

tirely in the supernatant fluid and only to a very slight extent in the crystals.

3. Coagulation of heparinized and hirudinized plasma. The results of mixing broth cultures of various strains of staphylococci with human plasma which had been collected by using heparin as an anticoagulant were essentially the same as with oxalated or citrated plasmas. Four milligrams of heparin were used for 10 cc. of blood. Ten strains of staphylococci which had been found to clot oxalated plasma likewise clotted that prepared with heparin and in approximately the same length of time.

Similar experiments were performed, using hirudin (10 mgm. to 10 cc. of blood) as the anticoagulant and the same results obtained.

TABLE II

	HUMAN PLASMA		RABBIT PLASMA		
	Strain FS	Strain Filb	Strain FS	Strain Filb	Strain SM
Original filtrate	2–16 hrs. (+)	2–16 hrs. (++++)	1 hr. (++)	1 hr. (++++)	25 min. (++)
Alcohol precipitate concentrated 10 times	1 hr.	15 min.	10 min.	15 min.	
Alcohol precipitate concentrated 20 times	15 min.		15 min.		11 min. (++++)
Alcohol precipitate concentrated 5 times					15 min. (++)
Alcohol precipitate unconcentrated					30 min. (++)

Five active strains of staphylococci were tested and were found able to clot plasma prepared in this way. Therefore, both heparinized and hirudinized human plasma showed the same degree of susceptibility to coagulation by broth cultures of staphylococci as did oxalated or citrated plasma. Gratia (6c) reported similar results when using hirudin.

4. Adsorption. Attempts were made to adsorb the coagulating substance from sterile filtrates with charcoal, kaolin, talcum and permutit, but the results were variable and the experiment was on the whole unsuccessful. However, when aluminum hydroxide was used as an adsorbent, the active filtrates lost their clotting power after shaking them with this substance and then centrifuging. At-

tempts to remove the coagulase from the aluminum hydroxide were, however, unsuccessful. Elution was attempted by the use of a phosphate buffer solution according to the method outlined by Garner and Tillett (17).

5. The coagulating activity of blood-broth cultures of staphylococci. A strain of staphylococcus which when cultivated in plain broth clotted plasma in 30 minutes at the fastest was grown in blood-broth (plain broth containing a very little rabbit's blood), and this preparation was found to clot the same plasma in 5 minutes. This acceleration of coagulation is not due to thrombin in the rabbit's blood, as the blood-broth itself will not clot plasma; nor to a combination of staphylococcus culture and thrombin from the blood, for if the culture and the blood-broth are mixed and then immediately put with the plasma, the clotting time is not shortened. It is not due to hemolyzed blood, for that hemolyzed by inactive staphylococcus or by streptococcus does not cause coagulation. Other strains of staphylococcus showed this same phenomenon with their blood broth cultures, but to a less marked extent.

6. Effect of bacteriophage. Bacteriophage was used in the hope that by breaking up the cell bodies of the staphylococci the plasma-coagulating substance would be enabled to pass through the filters. In 2 strains this result was obtained, and the broth cultures (whose filtrates were inactive), when cleared of the organisms by bacteriophage and then filtered through a Berkefeld candle, did clot plasma. In such cases as these it is assumed that the coagulating substance is intimately connected with the bacteria themselves, and then by breaking up the bacteria this substance is liberated into the medium in small enough particles to pass through the filter.

In the majority of strains of staphylococci, however, bacteriophage was found to exert quite a different influence on the coagulating property. By means of this substance moderate growths of staphylococci in broth could be cleared so that cultures of the fluid showed only a few scattered colonies, but this "bacteriophaged" culture had then lost much or at times all of its coagulating power. This test was done on about 10 strains, with the result that all (excepting the 2 mentioned above) showed either a marked inhibition of plasma-clotting or a complete loss of activity. In some of these clotting was much de-

layed and only took place when there was a secondary growth of the staphylococci in the plasma.

To test whether or not the phage had a direct inhibiting effect on clotting of plasma, a small amount of it was added to a broth culture of staphylococci just before it was mixed with plasma, and a good clot resulted in the normal length of time. An active phage (which could not clot plasma) was likewise added to a sterile filtrate of staphylococci, and coagulation of plasma was not inhibited even though the phage and the active filtrate were left together for periods of one hour, one day and three days before testing. We have no satisfactory explanation for this phenomenon in which cultural activity in some cases is inhibited by the dissolution of the bacteria by bacteriophage.

7. Coagulation by agar cultures. Agar cultures of staphylococcus aureus clot plasma with approximately the same speed and degree as do the broth cultures. Eleven strains of active staphylococci were tested. A small loopful of an agar culture of each was added to 0.5 cc. of plasma in sterile test tubes. Solid clots resulted in each case, usually in about 2 hours. Fibrinogen solutions are not coagulated as regularly nor as rapidly by this means as by the broth culture.

8. Coagulating contaminants. During the course of this investigation into the coagulating and fibrinolytic properties of staphylococci other bacteria were encountered from time to time—usually as contaminants—and these were also tested with plasma and fibrinogen, and with the clots of the same. Of 6 strains of B. subtilis, 2 were found capable of producing good (+++) clots with rabbit plasma but none with human. These did not coagulate rabbit nor human fibrinogen and their filtrates did not clot rabbit plasma. Of 3 strains of B. pyocyaneus, 1 clotted rabbit plasma fairly well (++), the other 2 were inactive and none clotted human plasma. Other organisms tested in like manner included Friedländer's bacillus, diphtheroid bacillus, pneumococcus type III and group IV, B. alkaligenes, micrococcus catarrhalis, B. coli, B. lactis aerogenes and streptococcus viridans. None of these possessed any coagulating activity.

COAGULATION OF FIBRINOGEN

Table III shows that of 24 strains of staphylococci capable of coagulating human and rabbit plasma, 17 also clotted human and 15

rabbit fibrinogen. The fastest time for clotting of *human* fibrinogen was 2 hours and the average time 3 hours (if we exclude one strain taking 16 hours). The time taken for coagulating *rabbit* fibrinogen was more rapid, the fastest time being 15 minutes and the average

TABLE III

Coagulation of fibrinogen

STRAIN	HUMAN FIBRINOGEN		RABBIT FIBRINOGEN	
	Culture	Filtrate	Culture	Filtrate
Pugh	2 hrs.	16 hrs.	2 hrs.	16 hrs.
201	2 hrs.	2 hrs.	2 hrs.	16 hrs.
GVS	2 hrs.	10 hrs.	2 hrs.	10 hrs.
FST	4 hrs.	17 hrs.	0	0
GFS	2 hrs.		15 min.	
Crom	16 hrs.	16 hrs.	0	0
VV	2¼ hrs.		0	
FS	2 hrs.		15 min.	
Sm	2 hrs.		15 min.	
PR	10 hrs.		2 hrs.	
Earl	2 hrs.		6 hrs.	
Dun	2 hrs.		0	
Rain	2 hrs.		2 hrs.	
Est	0	0	2 hrs.	0
Grif	2 hrs.	16 hrs.	2 hrs.	16 hrs.
Dub	4 hrs.		4 hrs.	
Tal	6 hrs.		6 hrs.	
Jack	3 hrs.	0	1 hr.	0
Smith	0	0	0	0
HLE	0		0	
V103D2	0	0	0	
Just	0		2 hrs.	
KT	0		0	
BS	0		0	
Number active....	17	6 (out of 9)	15	4 (out of 9)
Average time.....	3 hrs. (excluding 1, 16 hrs.)	13 hrs.	2¼ hrs.	14 hrs.

2¼ hours. On the other hand, these two fibrinogens are clotted with equal rapidity, in a few seconds, by thrombin. There is then a definite difference between the coagulation of fibrinogen and of plasma by staphylococci, the fibrinogen being clotted more slowly and in most

cases more incompletely than the plasma. It is likely that there are some substances in plasma but not in fibrinogen which aid in the clot production by staphylococci.

REACTIONS OF "ABNORMAL" PLASMAS

No extensive study was made to determine whether or not blood plasma obtained from patients suffering from various pathologic conditions reacted any differently from that of normals to this property of staphylococci, but we had the opportunity to study a few in this manner and it seems worth while to mention the following results. Staphylococci coagulated the following plasmas as well as that of normals: (1) Acute thrombocytopenic purpura (1 case); (2) Long-standing staphylococcus infections (3 cases); (3) Polycythemia vera (1 case)—was clotted slightly more rapidly than normal plasmas; (4) Hemophilia (2 cases)—these were clotted by staphylococci with speed equal to the normal, but their coagulation times with calcium were prolonged. (6) Staphylococcus septicemia with jaundice (2 cases): the clotting times of these plasmas (which were deeply bile-tinged) were markedly prolonged and solid clots were rarely formed by staphylococci or by calcium though thrombin coagulated it normally. This alteration in coagulability is probably due in part to the actual presence of the bile and in part to a change or diminution in the fibrinogen.

ANIMAL EXPERIMENTS

These experiments will not be given in detail as they were nearly all negative and did not seem to throw any real light on the possible significance of the coagulase reaction in human infections with staphylococci. Mixtures of staphylococcus cultures and plasma, in equal proportions—such mixtures clotting within 15 to 30 minutes in test tubes—were injected intracutaneously, subcutaneously and intrascrotally into rabbits, but no clots resulted.

Intrapleural injections were carried out with large amounts (20 to 30 cc.) of these same mixtures of plasma and staphylococci or plasma and potent filtrate, and the rabbits killed in 1½ to 3 hours. Not only did no clots result (except for very small clots in 2 of the 15 rabbits), but to our surprise the fluid did not coagulate afterward in test tubes,

and all tests for the presence of fibrinogen in it were negative.[1] Control injections of plasma or plasma and saline were made into the opposite pleural cavity and in 4 cases in which no bleeding occurred the fluid was likewise found no longer to contain any fibrinogen, though it had been subjected to nothing but 1½ to 3 hours' residence in the pleural cavity of a live rabbit. If the mixture of coagulase and plasma were injected into the pleural cavity of a dead rabbit or if the animal died within 15 minutes of the injection, coagulation did occur in the normal length of time. We have no adequate explanation to account for the loss of the fibrinogen under these circumstances. Sections made after carefully fixing the lungs in Zenker's and then staining for fibrin showed no appreciable amount of fibrin on the pleural surfaces. No signs of fibrin could be found grossly in the pleural cavities. It does not seem reasonable to postulate a selective absorption or destruction of the fibrinogen. Further studies are necessary to attempt to clarify this problem.

Intravenous injections were likewise carried out with potent cultures, using massive doses in an attempt to overcome the factor of dilution. Such animals did not show intravascular clotting either clinically, grossly or microscopically, though controls of corresponding proportions of blood and staphylococcus culture in test tubes coagulated readily.

DISCUSSION

In this paper we have presented evidence showing that the coagulase reaction is a very consistent property of staphylococcus aureus, though the speed and completeness of the reaction vary considerably in the individual strains. The bacteria-free filtrates of about half of the coagulating strains herein reported clotted plasma and a few clotted fibrinogen. Attention is called to the importance of being sure that the filtrate is free from calcium as that substance is washed through with the first few cubic centimeters of fluid in high enough concentration to clot plasma.

Except in an occasional strain, the whole broth culture was a more

[1] If bleeding occurred into the fluid because of injury to the lung during the injection (as it did in 7 of the 15 rabbits), no coagulation was found in the pleural cavity, but the fluid did clot spontaneously after being removed.

rapid coagulant than the filtrate, thus showing that even when the coagulase is excreted into the culture media some of it remains bound to the bacteria themselves.

In the case of some of the staphylococci it is evidently necessary for them to grow in the plasma or fibrinogen to clot them, but with others the broth cultures may be mixed with the plasma or fibrinogen and then put into the icebox immediately—thus inhibiting growth—and good clots result. It is especially the strains in this latter group whose sterile filtrates can coagulate plasma.

The fact that the coagulating substance can be precipitated out of the filtrate by alcohol and then by concentration in saline increased in speed of reaction by 5 to 10 times suggests the possibility of its practical application as a coagulant.

During this study at least 30 additional strains of Staphylococcus aureus were isolated from human infections due to that organism and they all possessed the plasma-clotting property. Filtrates of some of these were likewise active.

No especial attempt has been made in this work to link up the coagulating activity of these staphylococci with their pathogenicity. Most of the active strains did come from pathologic sources but some laboratory contaminants were likewise found to be capable of producing clots. However, the majority of inactive strains studied were taken from minor infections in which the organisms seemed to exhibit very little virulence, and we can conclude that a strain of staphylococcus aureus is probably of little importance from a pathogenic standpoint when its broth culture fails to coagulate plasma. We are inclined to agree with several of the authors mentioned earlier that the "coagulase" reaction is a useful indication of the staphylococcus's pathogenicity, at least to this degree: if a pathologic lesion yields a staphylococcus aureus whose culture is hemolytic and coagulates plasma, that strain of staphylococcus is pathogenic. The following data also make it seem likely that pathogenic strains of staphylococci are more apt to exhibit the clotting reaction than others. Of the 26 plasma-coagulating strains here reported, 15 were studied by Burkey (17) for their rabbit-killing properties. He found that 6 of these 15 were toxin-producers (*i.e.*, killed a rabbit by intravenous injection in 1 to 2 days) and the others killed the rabbits more slowly, producing

multiple abscesses. Of the 8 strains which we found did *not* coagulate plasma, Burkey studied 7 in this way and none were toxic or pathogenic to rabbits on intravenous injection.

We do not know whether or not this property of staphylococci has any real significance in human infections with this group of organisms. The theory has been entertained at times that the plasma-coagulating property of staphylococci may help to explain the tendency of infections with those organisms to remain localized, in contrast to the rapid spread of streptococci from the point of inoculation. It does not seem reasonable, however, to presume that such a reaction plays any important rôle in localized staphylococcus lesions, for low-grade staphylococcus albus infections are even more apt to remain localized, and yet cultures of those organisms rarely exhibit the coagulase reaction. Moreover, Menkin and Walston (19) have recently shown by injecting the coagulase fraction of staphylococcus filtrate into the tissues with trypan blue that there is no fixation of the dye, whereas injection of the whole filtrate with the dye does result in fixation. They conclude from this work that the localization of infection with this organism is independent of the plasma-clotting reaction.

Animal experiments with staphylococcus cultures and filtrates—which were almost entirely negative—and the absence of any evidence of excessive intravascular clotting or thrombosis in a limited number of autopsy reports of staphylococcus aureus septicemias[2] gives added weight to our conviction that the coagulase reaction is mainly a test-tube phenomenon which cannot be used to explain any of the peculiarities of infections with that organism. Thrombi are commonly found in the small veins in the neighborhood of any infection, but it is not

[2] Twenty-seven cases were studied, and in one there were "rather conspicuous masses of fibrin" in a few veins of the liver, but otherwise no signs of abnormal clotting. There were no thromboses in any of the large veins away from the site of the original infection. In an approximately equal number of cases of pneumococcus and of hemolytic streptococcus septicemias there was found in 1 of each thrombosis of a large vein. One additional very recent case of staphylococcus aureus septicemia should be mentioned, however. In this patient, at autopsy, both iliac and femoral veins were found occupied by large clots and there were emboli in pulmonary vessels. The portal of entry for the infection was not definitely determined. This staphylococcus exhibited the coagulase reaction in the test tube, but did not clot plasma any more rapidly than the average.

apparent from autopsy reports that the staphylococcus aureus has any unique potency in eliciting clots or thromboses in the vessels surrounding its lesions.

We cannot give any definite explanation for the apparent impotence of the coagulase in the human body, but the high dilution in which it must occur in the blood stream and the possibility of rapid excretion through the kidneys are factors which must be borne in mind. It is also quite possible that an "anti-coagulase" is formed in the body during infections with staphylococci.

SUMMARY

1. Of 34 strains of staphylococci from various sources studied for their plasma-coagulating properties, 26 clotted plasma and 17 clotted fibrinogen.

2. In testing at least 30 additional strains of staphylococcus aureus from various types of human staphylococcus infections, every one was found to exhibit this reaction with plasma.

3. The active ones were all aureus varieties except 1, and all were hemolytic except 1. All but 1 of these strains were obtained from pathologic lesions.

4. The inactive ones were usually albus, non-hemolytic, and were encountered mainly as contaminants or secondary invaders.

5. The bacteria-free broth culture filtrates of 14 of the 26 active strains likewise coagulated plasma and 6 of these coagulated fibrinogen also.

6. The active material could be precipitated from the filtrates by alcohol and then obtained in a saline solution. This could be concentrated by dissolving in smaller amounts of saline.

7. From this study and from observations of many in the literature, the conclusion seems justified that the coagulation reaction is a reliable sign of pathogenicity if considered in conjunction with the hemolysin production and the virulence toward animals. A strain of staphylococcus aureus which does *not* clot plasma is almost certainly not pathogenic.

8. We cannot draw any definite conclusions as to the bearing this property of the staphylococcus has on human infections with this group of microörganisms. Attempts to reproduce the blood clotting

phenomenon in rabbits by intravenous, intrapleural and other routes of injection were almost entirely unsuccessful, and thrombosis or intravascular clotting are not found to be outstandingly common in human cases of staphylococcus aureus bacteremia.

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THE FIBRINOLYTIC PROPERTIES OF STAPHYLOCOCCI

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In a recent report (1) we have presented a study of the plasma-coagulating properties of staphylococci. In addition to this reaction which is produced by most staphylococcus aureus strains and by practically all of the pathogenic ones, a few strains also exhibit the power of dissolving fibrin, though this reaction takes place quite slowly. This paper deals with the results of an investigation of the phenomenon of fibrinolysis as observed in some of the plasma-coagulating strains referred to in the earlier paper.

LITERATURE

Much (2), in 1908, was the first to call attention to the fibrinolytic property of staphylococci. He found that it took place most often in the tests in which the staphylococci had caused a slower and less complete coagulation.

Kleinschmidt (3) concluded that there was a "fibrinolysin" in some staphylococcus cultures, causing the clotted plasma to become redissolved; that the reaction, however, was a matter of days, and was only brought about by living organisms. He also reported that, if a weak solution of phenol were added to plasma which had undergone clotting and lysis by staphylococci, the clot quickly reformed. It may be briefly stated here that all of our attempts to repeat this phase of Kleinschmidt's experiments were unsuccessful. The dissolution of fibrin by staphylococci appears to be an irreversible reaction, since all attempts to recoagulate the solution have been unsuccessful.

This reaction was also mentioned by v. Gonzenbach and Uemura (4) and more thoroughly investigated by Gratia (5) in 1921, who found staphylococci capable of redissolving the plasma-clots they had produced. The speed of this reaction varied with the species of animal from which the plasma was obtained. With dog plasma, solution of the fibrin took place 2 to 3 hours after coagulation; while human plasma took much longer both for clotting and fibrinolysis. Plasma which had been clotted and dissolved in this way no longer contained fibrinogen and was even found to be capable of preventing the coagulation of plasma by calcium or thrombin.

Aoi (6), in 1932, reported the results of an exhaustive study of the fibrinolytic properties of staphylococcus aureus. He found that three reactions of the staphylococcus—hemolysis, "kinase" (*i.e.* plasma coagulation) and fibrolysis—are intimately connected with their pathogenicity as determined by skin tests on rabbits. He was able to obtain the fibrinolytic substance by filtration, finding that the first fluid to come through the filter does not contain it—evidently because it is adsorbed by the filter—but that if more is filtered, it can be obtained in the filtrate. The fibrolysin resists heat (100° for 1 hour), direct sunlight and many antiseptics. It occurs with greatest regularity in 5- to 14-day-old cultures and may be precipitated out by 75 per cent alcohol or by acetone. Either the acetone precipitate or the autolysate itself may be used as an antigen—injected into the ear vein of a rabbit weekly—and the plasma of a rabbit so immunized was found to inhibit fibrolysis.

Gengou (7) (1933) found that the coagulating substance which he obtained in a bacteria-free solution also had the power of dissolving the clots it had produced and this fibrinolytic substance withstood the same high temperature (100° for fifteen minutes) that the coagulating substance was able to withstand. Fibrinogen may likewise be clotted by this substance, but becomes redissolved much more rapidly than plasma-clots, for the globulin and especially the albumin in plasma have an "anti-lytic" effect.

Vanbreuseghem (8, 9), in two articles in 1934, reported some interesting studies on the solubility of the fibrin formed by the action of the "staphylocoagulase" on ascitic fluid and on plasma. He found that the fibrin was easily dissolved in alkaline distilled water, regelified by adding a trace of acid, and then dissolved again in an excess of acid. Some plasma clots are not re-dissolved in this way, for if oxalated rabbit plasma is clotted by calcium or thrombin, the clot is not dissolved by alkalinized distilled water. (We were not able to confirm these findings in our own studies, for when slightly alkaline distilled water was added to a clot of human or rabbit plasma that had been coagulated by staphylococci, the fibrin was never entirely dissolved, and acidifying it with dilute acetic acid did not cause the clot to be reformed. Our own experiments on fibrinolysis, as will be seen, show clearly why we could not expect to obtain the same results as the above author. We find that the lysis which occurs in plasma-staphylococcus clot represents an irreversible reaction in which the fibrinogen is destroyed or totally changed in nature. Others have found the same to be true.)

In a later report Vanbreuseghem (12) confirmed the observations of Gengou (7), who had found that some strains of staphylococci destroy fibrinogen without coagulating it. He was able, however, to retard this reaction so that coagulation took place. This could be accomplished either by carrying out the tests at a temperature of 20° rather than the usual 37°, or by adding platelets to the fibrinogen-coagulase mixture.

Madison (13) studied 145 strains of Staph. aureus and albus for their fibrinolytic reactions and made titrations of this property. Twenty-four hour broth cultures in dilutions were added to human fibrinogen and the mixture clotted by thrombin.

He found that 77 per cent of the 78 strains from *superficial* human infections had *no* fibrinolytic function, whereas 90 per cent of 30 strains from "internal" human lesions (septicemia, empyema, etc.) were fibrinolytic. None of 24 veterinary strains was fibrinolytic. Sera containing the antifibrinolysin for streptococci did not neutralize the staphylofibrinolysin.

METHODS OF STUDY AND RESULTS

The fibrinolytic properties of staphylococci and their filtrates were studied in the following ways: (1) their ability to redissolve the clots they have formed with plasma or fibrinogen, and (2) their dissolution of clots formed by the addition of calcium or thrombin to mixtures of plasma and culture. Mixtures of fibrinogen and culture were similarly clotted with thrombin. In the latter instances the coagulating agent was added before the culture action took place.

Lysis of plasma clots

a. Certain strains of staphylococci which are capable of forming a clot when their broth culture is placed with plasma also have an additional property, the ability to dissolve that clot—the power of fibrinolysis. This property is not limited to staphylococci, for in the past three years Tillett and Garner (10) have shown that hemolytic streptococci under various conditions exhibit this power to a much more marked degree. In the case of the streptococci, the fibrinolysis of plasma-calcium clots takes place in from a few minutes to a few hours, whereas with the staphylococci the ones which possess the fibrinolytic activity are able to dissolve the clots only after many hours or days.

The same methods which were used for the investigation of the staphylococcus' coagulating properties were used for determining its fibrinolytic activity: 0.5 cc. of broth culture (18 hours to 1 weeks' growth, usually) was added to 0.5 cc. of plasma, diluted 1:5. This mixture was then allowed to go through its cycle of clotting and lysis in a small sterile test tube at room temperature or at 37°C., the results being similar at either temperature. The readings were made after lysis was complete. A summary of the results of this study may be found in Table I, where it will be noted that of the 26 strains of staphylococci which clotted plasma, as tabulated in our earlier paper, only 7 could also redissolve the clot. No staphylococci were found

which exhibited the fibrinolytic and not the plasma-clotting activity. As mentioned above, the fibrinolysis brought about by these organisms was not a rapid one, for the fastest lysis for human plasma clots

TABLE I

Fibrinolysis by coagulating strains

(Time of dissolution of self-produced clots)

STRAIN	PLASMA CLOTS		FIBRINOGEN CLOTS	
	Human	Rabbit	Human	Rabbit
Pugh	0	0	12 hrs.	24 hrs.
201	0	0	0	0
GVS	0	0	0	0
FST	0	0	24 hrs.	
GFS	0	0	10 hrs.	4 hrs.
Crom	0	0	48 hrs.	
VV	0	0	24 hrs.	
FS	0	0	24 hrs.	36 hrs.
Sm	0	0	12 hrs.	12 hrs.
PR	0	0	40 hrs.	12 hrs.
Earl	0	0	24 hrs.	36 hrs.
Dun	0	0	40 hrs.	
Rain	0	0	10 hrs.	10 hrs.
Est	0	0		24 hrs.
Grif	0	0	24 hrs.	24 hrs.
Ha/R	0	0		
Dub	0	0	15 hrs.	20 hrs.
Tal	0	0	10 hrs.	10 hrs.
Ha	0	7 days		
Jack	0	0	18 hrs.	48 hrs.
Smith*	4 days	4 days		
HLE	4 days	4½ days		
V103D2	2½ days	3 days		
Just	4 days	5 days		8 hrs.
KT	5 days	6 days		
BS	3 days	7 days		
Average time.....	4 days (3.7)	5 days (5.2)	22 hrs.	21 hrs.
Number of fibrinolytic.....	6	7	15	13

* Average time of fibrinolysis in many tests used here.

took place in 30 hours ("Smith"), and the average was about 4 days. The most rapid lysis of rabbit plasma clots was 2 days and the average approximately 5 days. The remaining strains produced incom-

plete or no lysis and the clots were discarded after observing them for 10 days to 2 weeks. The same strains which showed this activity toward human plasma clots also dissolved the rabbit plasma clots and, in general, in approximately the same length of time. In short, the majority of staphylococci in this series showed no fibrinolytic power toward *plasma* clots under these experimental conditions, and the few which did dissolve the clots did so consistently but very slowly.

b. Another method of studying this fibrinolytic activity was to clot the plasma-staphylococcus mixture (0.2 cc. plasma, 0.8 cc. normal saline and 0.5 cc. culture) with calcium and observe the length of time required for lysis. (This was the technique used by Tillett and Garner in similar studies with streptococci.) The calcium used was 2 to 4 drops of a dilute sterile solution of calcium chloride. If the cultures were not over 2 to 3 weeks old and within the required limits of pH (6.4 to 8.0) a clot resulted in a few minutes. The same method was applied to plasma clotted by thrombin and the results in the two series were found to run nearly parallel. It was found that the same strains (with an occasional exception) which showed some fibrinolytic activity in the self-produced clots were also capable of dissolving the clots formed by adding calcium or thrombin to plasma, though the process in most cases was slower by the latter method.

c. Fibrinolytic activity of the sterile filtrate: The fibrinolytic property of the staphylococci appeared to be, in all of our strains except two, inseparable from the live organisms, and presumably they elaborated it as they grew in the clot. Some other workers were able to obtain this substance in bacteria-free filtrates, however, and so it may be that as more strains are studied, more broth filtrates will be found to contain the fibrinolytic element. The filtrate of one strain ("Smith"—the one whose culture was the most actively fibrinolytic) was able to dissolve a human plasma-calcium clot, and another ("H. L. E.") also showed this same reaction with human plasma clots, but more irregularly. (It will be shown later, however, that the fibrinolytic substance may be obtained from the filtrates of other strains [*e.g.*, "B.S."] by precipitation with alcohol.)

Growth of the staphylococci in the clot does not appear to be the only requirement for the production of lysis, for it was found that

loops of plain agar cultures of three of the fibrinolytic strains, though they clot plasma in 2 hours, fail to dissolve these clots. This would seem to indicate that either the growth in *broth* is necessary for the production of the fibrinolytic material, or that there are substances in broth which aid fibrinolysis by these organisms.

Lysis of fibrinogen clots

This was studied by the same method as was employed with plasma: by determining the speed of liquefaction of the clots produced by the action of the staphylococci themselves on the fibrinogen, and also the speed with which the fibrinogen-staphylococcus-thrombin clots were dissolved. In general this reaction takes place very much more rapidly and with many more strains than the similar reaction with plasma clots. The majority of the strains dissolve the clots they have formed in a few hours, but some remained solid for days or even for a week or more. One fact that makes it hard to evaluate this fibrinolysis is that fibrinogen clots have a tendency to spontaneous lysis. This occurs with both human and rabbit fibrinogen after clotting with thrombin. This occurs quite frequently, and when it does, the clots become liquid again in 1 to 4 days. Such a reaction takes place even when all the materials employed are sterile, thus proving that the liquefaction is not due to the presence of any bacteria. Consequently, it is hard to conclude that the staphylococci have any great fibrinolytic power unless they dissolve the clot much more quickly than when it often dissolves spontaneously. Of the 17 strains of staphylococci capable of coagulating human fibrinogen, 15 are able to redissolve the clots (Table I). Of the 15 strains that clot rabbit fibrinogen, 13 also liquefy the clots they have produced. The most rapid lysis of human fibrinogen clots was 10 hours and the average time 22 hours. The fastest for rabbit fibrinogen was 4 hours and the average 21 hours.

In another series of tests the fibrinolytic power of the various strains of staphylococci was studied by placing a small amount of the broth culture in a tube with fibrinogen, clotting the mixture with thrombin and then observing the time of lysis of the clots. The proportions were: fibrinogen 0.2 cc., saline 0.8 cc., staphylococcus culture 0.5 cc., thrombin (sterile) 2 drops. A control was used of saline instead

of the culture. Of 19 strains tested in this way (all of which were capable of clotting plasma, many also able to clot fibrinogen) 14 showed significant lysis of human fibrinogen clots (*i.e.*, in less than 24 hours), and 13 (not all the same ones) dissolved rabbit fibrinogen clots prepared in this way. The most rapid lysis of human fibrinogen clots took place in 5 hours, the average time being 8 hours; for rabbit the fastest was 4 hours and the average 10 hours. A series of controls was set up at the same time and according to the same technique, but in these we used staphylococcus cultures which were unable to coagulate plasma or fibrinogen. These control clots showed no signs of becoming dissolved in a week.

Another series was studied in which less than one-half as much fibrinogen was used. (The same amount as used by Tillett and Garner with streptococci.) The results are comparable to the above in which more fibrinogen was employed but the reaction took place more rapidly. The most rapid dissolution of human fibrinogen clot took 2 hours and of rabbit, 1½ hours; the average time being 5½ and 4.7 hours, respectively. (The fibrinolytic strains were included in these experiments with thrombin—they do not coagulate fibrinogen spontaneously—and were found to dissolve these clots only slightly more rapidly than the other strains.)

DISCUSSION OF LYTIC ACTION OF STAPHYLOCOCCI ON FIBRINOGEN

Strains of staphylococci which are incapable of coagulating plasma or of dissolving clots are likewise inactive in these respects toward fibrinogen solutions. Some strains which are able to coagulate fibrinogen can also dissolve the clots they have produced, and there are some which do *not clot* fibrinogen but *do dissolve* fibrinogen clots that have been produced by means of thrombin. Some of the best plasma-coagulating strains show no coagulating activity toward fibrinogen. This may mean either that there is some substance in plasma and not in fibrinogen which aids in coagulation, or that these staphylococci may dissolve the fibrinogen before they clot it. This theory was substantiated by the fact that only one of the 7 strains which we found to show fibrinolytic activity toward plasma clots could clot fibrinogen (that one clotted rabbit but not human fibrinogen). Additional evidence was obtained from some experiments in which the cultures of

these particular strains were mixed with fibrinogen and then put in a water bath at 37°C. If these mixtures were then tested with thrombin after one-half hour they clotted and if heated to 56°C. for a short time they showed flocculation—indicating that the fibrinogen was still intact. However, if left in the water bath 16 hours and then tested with heat or thrombin, the reactions for fibrinogen were not obtained—showing that it had been altered or destroyed without going through the stage of coagulation. A control solution of fibrinogen and saline left in the water bath for the same length of time showed normal reactions for fibrinogen when tested with thrombin or heat. Two other controls were used—strains of staphylococci having no coagulating nor lytic activity for plasma or fibrinogen—and tested in a similar manner; the fibrinogen was found to be intact after 16 hours. It seems likely, then, that some staphylococcus cultures which exhibit definite fibrinolytic activity toward plasma clots, also have the property of being able to break up or destroy fibrinogen in solution before it has been coagulated.

Fibrinogen clots which had been liquefied by staphylococci were put into a water bath at 56°C. for 1 to 2 hours with no change in their appearance. (A weak fibrinogen solution treated in the same manner will show marked flocculation.) If it is treated with thrombin no clot reappears. The fibrinogen has either been destroyed in this process of lysis or markedly altered. Spontaneously lysed fibrinogen clots likewise fail to react to heat or thrombin, indicating that destruction of the fibrinogen can occur without the aid of bacteria.

FIBRINOLYSIS BY ALCOHOL PRECIPITATE OF FILTRATES

It has been mentioned that the fibrinolytic substance (in these observations) rarely is found in the bacteria-free filtrate. However, by alcohol precipitation of the coagulase from the filtrate and concentration of the precipitate in normal saline, we not only are able to obtain the *coagulating* substance in a much more potent solution than that found in the original filtrate or in the unconcentrated alcohol precipitate, but we likewise can obtain an active *fibrinolytic* substance in this manner. That this fibrinolytic substance can likewise be concentrated by dissolving the alcohol precipitate in smaller amounts of saline is illustrated by Table II, which demonstrates the increased

potency of concentrated specimens of precipitate in respect to both coagulating and fibrinolytic properties.

These dissolved clots when treated with thrombin do not become recoagulated. Many other similar experiments have supported the findings illustrated by this table.

FIBRINOLYTIC CONTAMINANTS

The fact that staphylococci are not the only fibrinolytic bacteria was mentioned earlier, and the work of Tillett and Garner (10) demonstrating the rapid fibrinolysis brought about by hemolytic streptococci has been referred to. During the course of this study we found several other species of bacteria—encountered as contaminants—which exhibited this property in a more marked degree than most of

TABLE II

Coagulation of plasma and fibrinolysis by alcoholic precipitates of filtrates

STRAIN AND CONCENTRATION	HUMAN PLASMA		RABBIT PLASMA	
	Coagulation	Lysis	Coagulation	Lysis
"Smith"—concentrated 20 times	12 min.	7 hrs.	12 min.	2½ hrs.
"Smith"—concentrated 5 times	15 min.	7 hrs.	15 min.	7 hrs.
"Smith"—broth culture filtrate	0		30 min.	0 (4 days)
BS—concentrated 20 times	15 min.	3 days	15 min.	7 hrs.
BS—concentrated 5 times	50 min.	3 days	35 min.	16 hrs.
BS—broth culture filtrate	3 days	0	50 min.	0 (4 days)

the staphylococci had done. Five strains of B. subtilis dissolved plasma-calcium clots in 1 to 6 days. Other organisms showing this fibrinolytic power were B. proteus (2 days), B. pyocyaneus (3 days), diphtheroids (4 to 5 days); and one strain of B. alkaligenes dissolved a rabbit plasma-calcium clot in 20 hours. Streptococcus viridans, Friedländer's bacillus and the pneumococcus did not produce fibrinolysis. These findings with contaminating organisms are mentioned because they serve to emphasize the importance of observing strictly sterile technique when studying the fibrinolytic property of staphylococci. These results also bring into question the importance of the fibrinolytic reaction of the staphylococcus, which by many has been considered a property limited to that variety of organism and often thought to be a good measure of its pathogenicity.

DISCUSSION

A small proportion of the plasma-coagulating strains of staphylococci in this series were found to possess the property of slowly dissolving fibrin in plasma clots. The majority showed a definite fibrinolytic activity toward fibrinogen clots—either self-produced ones or those formed with thrombin. The strains which coagulated plasma and then dissolved the clots demonstrated their fibrinolytic potency toward fibrinogen solutions by destroying the fibrinogen without coagulating it. The increased susceptibility of fibrinogen clots to dissolution by staphylococci may be explained by the inhibiting action of albumin and globulin in plasma—as suggested by Gengou. A fibrinolytic substance could be obtained from the bacteria-free filtrates of some strains, at least, by precipitation with alcohol, and the speed of its reaction in self-produced clots multiplied markedly by concentrating the precipitate. The fibrinolytic substance, as observed in the study of this group of staphylococci, was not a very potent one in comparison with that of the hemolytic streptococcus, even when concentrated following precipitation. With the streptococci the lysis occurs in a very few minutes, in contrast to the slow process as brought about by the staphylococci, for which hours, or more often, days are required.

It did not seem entirely clear whether or not the staphylococci produced lysis of fibrinogen and plasma clots by a specific lysis of the fibrin or merely by utilizing the fibrin for purposes of growth. Two facts, however, make it seem likely that certain strains do produce a specific fibrinolysin: first, that though all staphylococcus strains may grow in the clots, only a few of them dissolve the plasma clots and there are variations over a wide range of time in their lysis of fibrinogen; and second, that a fibrinolytic substance may be obtained free from the bacteria by alcoholic precipitation of sterile filtrates.

We do not know whether or not this property can have any significance in actual infections with these organisms. Of the 7 fibrinolytic strains 5 were tested by Burkey (11) for their rabbit-killing properties; 3 were toxin-producers—killed the animal within 24 hours after intravenous injection of a 10-day broth culture; 1 killed

a rabbit in 3 days, producing multiple abscesses, and 1 was not pathogenic for rabbits. This series is too short to warrant any definite conclusions regarding the parallelism between this property of staphylococci and their pathogenicity for rabbits.

It seems reasonable to assume that human infections with organisms having this property may be more dangerous than with others if we consider the possibility of their leading to dissolution of fibrin-barriers and so a spread of the infection. It is conceivable, likewise, that a thrombophlebitis—in the neighborhood of a staphylococcus infection, for example—is more of a menace when infected with staphylococci which are capable of dissolving the fibrin and so releasing emboli into the circulation, than if the infecting organism is not fibrinolytic.

SUMMARY

1. In a study of a group of 26 staphylococci—all of which coagulate plasma—7 were found to dissolve plasma clots and 15 dissolved self-produced fibrinogen clots. These reactions were quite slow, averaging 4 days for plasma and 21 hours for fibrinogen.

2. Fibrinogen is destroyed in solution, without going through the process of coagulation, by the 7 strains which dissolve plasma clots.

3. The much greater susceptibility of fibrinogen to this reaction as contrasted with that of plasma is probably due to the lysis-inhibiting action of albumin and globulin in plasma.

4. The occurrence of spontaneous lysis of fibrinogen-thrombin clots makes us question the importance of the dissolution of fibrinogen clots by staphylococci, though the latter reaction is a more rapid one.

5. No straphylococci were found capable of fibrinolysis toward plasma or fibrinogen which did not possess the coagulating property.

6. The fibrinolytic substance could be obtained free from bacteria by alcoholic precipitation of the broth-culture Berkefeld filtrate in several strains. The speed of fibrinolysis could be markedly increased by concentrating this precipitate in smaller amounts of saline.

7. The possible significance of this property of some staphylococci in human infections with those organisms is discussed.

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PECULIAR GRANULES IN THE CELLS OF THE LIVER AND ADRENAL IN INFECTIONS

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In pathological sections of the human liver and adrenal cortex, fixed in formalin or Zenker's fluid and stained with hematoxylin and eosin, there appear in the cytoplasm oval, rod-shaped, or club-shaped granules dark with hematoxylin. Their average size is about 1 x 3μ, but they vary greatly. Their outlines are irregular. They may bulge out into ill-defined expansions. All the cells may be thickly dotted with them, or there may be only three or four granules in an occasional cell. In the cells of the adrenal cortex they may be fewer than in the liver cells. They are stained red with pyronin.

They have no special station in the lobule of the liver or in the adrenal cortex. Within the cells they are often indiscriminately arranged. At times they appear to be lined up in bands near the periphery, leaving a clear zone in the middle of the cells. Not infrequently they give the impression of pointing from the periphery toward the center of the cell in parallel rows. This pattern has nothing to do with the position of the nucleus. When it is eccentric, it may touch them. Or it may lie more centrally in the clear zone.

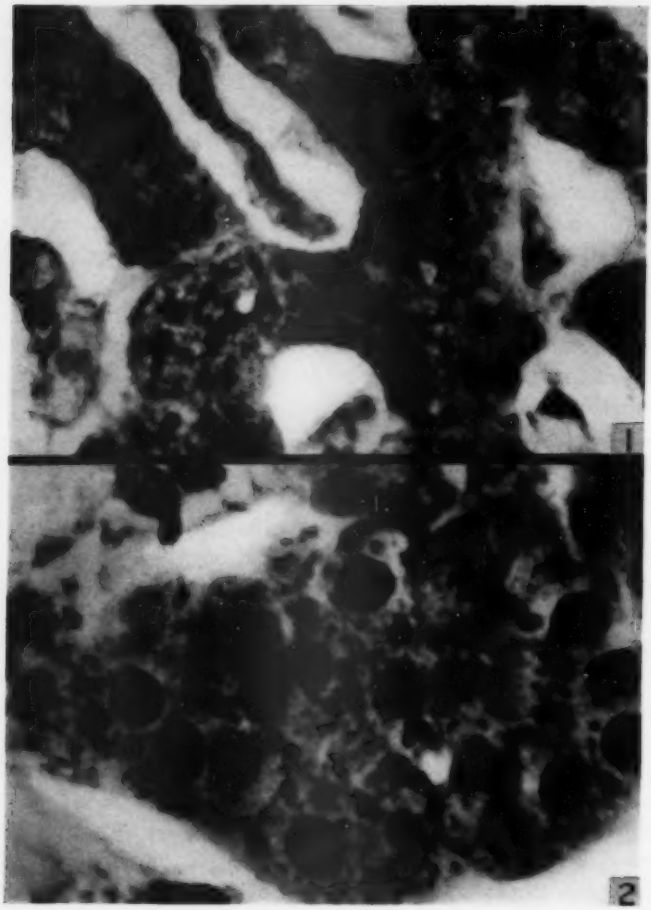
Since the granules occur very frequently and are quite distinct with the high, dry objective (Figs. 1 and 2), they must have been seen by many observers. And yet little or no mention of them has been made in the literature.

The following method was adopted for studying their occurrence in the cells of the liver. From 1000 successive autopsies performed at the Johns Hopkins Hospital during the years 1931 to 1933,[1] there were omitted all deaths below the age of 20, all cases not represented by slides in the files at the time, and all those in which the liver

[1] Autopsies No. 12000–12999 in the Johns Hopkins Hospital collection.

cells were too necrotic or too full of fat for study. The 406 cases that remained were classified as follows:

0—No granules apparent in the liver cells on cursory examina-



Autopsy No. 12023. Fig. 1, liver. Fig. 2, adrenal. The large black masses are nuclei. The smaller ones are the granules. Hematoxylin and eosin. × 1150. Photographs by Milton Kougl.

tion. Careful search may reveal three or four in isolated granule-containing cells here and there.

+—Granules distinct in all fields containing well preserved liver cells. About a dozen may be counted in almost any cell.

++—Cells thickly dotted with dark granules, 12–20 in a cell.

At first the more obvious method of classification, indicating only the presence or absence of the granules, was tried. It appeared to have no practical use. For, in sections that on reasonable study show no granules at all, a few may be found on minute scrutiny. After all, there always remains the possibility that a few might have been found in a section cut elsewhere. We were concerned here rather with the marked increase of the granules in certain conditions.

The result of a final rejudication, made after repeated study of the sections, was:

TOTAL	++		+		0	
	Number	Per cent	Number	Per cent	Number	Per cent
406	68	17	141	35	197	48

From observations made in the course of this work it was evident that no correlation could be made between the occurrence of the granules and the standard pathological changes of the liver, such as central necrosis, cloudy swelling, and the appearance of hemosiderin. Necrotic cells do not show the granules, but neighboring healthy ones may or may not. The granules are not stained by the usual stains for iron. In ordinary hematoxylin and eosin preparations they are easily distinguished from hemosiderin even within the same cell. Cloudy swelling neither insures their presence nor prevents it.

There is likewise no relation between the number of granules and the time elapsing between death and autopsy, which was, as a rule, between two and twelve hours, provided that the tissue is well preserved. The state of nutrition of the subject as measured by the weight/height ratio of the body is also without influence.

The granules appear somewhat less frequently in old people, but this observation is accounted for by factors other than mere age. Dr. A. R. Rich suggested on the basis of casual observations that they

appear best in intense infections. In spite of the exceptions noted in the tables below, this relation is as well established as the deficiencies of the method permit one to expect. It may not be the final connection. It may not be even immediate. But certainly it exists.

In attempting to analyze the cases from the standpoint of the relation of infection to the occurrence of the granules, the following scheme facilitated classification.

1. Cases in which an infection is the main cause of death.
2. Cases in which an infection is at least a contributory cause.
3. Cases in which an infection played no rôle in causing the death of the patient, although a minor or terminal infection was found.
4. Cases in which there was no reliable evidence of any infection, although in some there were grounds for suspecting the presence of infection.

Three specific infections which tend to run a chronic course were listed separately for special study, namely:

5. Cases in which tuberculosis was the main cause of death.
6. Cases in which the lesions of syphilis were the main cause of death.
7. Cases in which the lesions of rheumatic fever were the main cause of death.

To avoid the effects of prejudice, the slides were studied and classified according to the presence or absence of the granules with no previous consideration of the type of case concerned. The cases were then classified in the above manner purely on the basis of the clinical and pathological findings listed in the protocols and with no reference to the histological finding of the granules. The conclusions reported here were reached by a comparative study of these two independent classifications.

Analysis of the cases of tuberculosis shows that the ones with granules are, as a rule, the caseous fulminant forms, while the chronic, fibroid forms usually do not show them. Syphilis and rheumatic fever are characteristically negative. Four of the eight cases of syphilis and both cases of rheumatic heart disease in which granules were found were complicated by other infection.

The cases most regularly falling into the ++ class are severe

infections, such as septicaemia resulting from infected abortions, ruptured appendices, etc. All of ten cases of bacterial endocarditis showed granules. The occurrence of the granules is inconstant in lobar pneumonia, as well as in lobular pneumonia and the minor genitourinary infections. Four of five cases of bichloride of mercury poisoning were rated ++. This phenomenon may be due to the intense colitis provoked. Acute alcoholism, arsphenamine poisoning, acute yellow atrophy, illuminating gas poisoning, and hydrochloric acid poisoning were negative.

TABLE I

Relation of the granules to infection

	TOTAL	DEGREE TO WHICH THE GRANULES APPEARED					
		++		+		0	
		Number	Per cent	Number	Per cent	Number	Per cent
Infection* main cause of death	114	45	39.5	50	43.8	19	16.7
Infection* accessory cause of death	54	7	12.9	35	64.9	12	22.2
Total	168	52	30.9	85	50.6	31	18.5
Tuberculosis main cause of death	24	5	20.8	7	29.2	12	50
Syphilis main cause of death	29	2	6.9	6	20.7	21	72.4
Rheumatic heart disease	9	1	11.1	1	11.1	7	77.8
Total	62	8	12.9	14	22.6	40	64.5
Coexisting minor infection	96	4	4.2	33	34.4	59	61.4
No evidence of infection	80	4	5	9	11.3	67	83.7
Total	176	8	4.5	42	23.9	126	71.6

* Other than tuberculosis, syphilis, or rheumatism.

The chemical nature, origin, and significance of the liver and adrenal cell granules are obscure at present. The only structures I can find with which they might be identified are the so-called "protein storage" granules investigated by W. Berg (1). Like Berg's granules they stain with pyronin according to the method of Unna and Pappenheim (2). They are still better stained by more concentrated solutions of pyronin alone.

Nevertheless, from a morphological standpoint, they do not entirely resemble the viscous droplets shown in Berg's pictures. Dr.

I. Gersh of the Department of Anatomy kindly showed me a slide made from the liver of a rabbit, to which he had fed an egg four

TABLE II

Some diseases usually showing granules

	TOTAL	DEGREE TO WHICH THE GRANULES APPEARED					
		++		+		0	
		Number	Per cent	Number	Per cent	Number	Per cent
Septicaemia	47	24	51.1	17	36.2	6	12.7
Peritonitis	36	13	36.1	13	36.1	10	27.8
Infected wounds	24	7	29.2	13	54.2	4	16.6
Pulmonary abscesses	10	5	50	4	40	1	10
Pyelonephritis	27	10	37	13	48.2	4	14.8
Lobar pneumonia	10	1	10	4	40	5	50

TABLE III

Diseases usually without granules

	TOTAL	DEGREE TO WHICH GRANULES APPEARED											
		++				+				0			
		Total		With severe infection	With minor or no infection	Total		With severe infection	With minor or no infection	Total		With severe infection	With minor or no infection
		Number	Per cent	Number	Number	Number	Per cent	Number	Number	Number	Per cent	Number	Number
Tumors	62	5	8	3	2	20	32.3	6	14	37	59.7	4	33
Hypertensive heart disease	30	0	0	0	0	8	26.6	2	6	22	73.4	0	22
Coronary disease	14	1	7.1	0	1	2	14.3	1	1	11	78.6	0	11
Arteriolar nephritis	20	0	0	0	0	7	35	3	4	13	65	2	11
Cerebral hemorrhage	11	0	0	0	0	2	18.2	0	2	9	81.8	0	9
Injuries, surgical and accidental, not infected	22	1	4.5	1	0	8	36.4	3	5	13	59.1	2	11
Leukaemia	5	0	0	0	0	3	60	2	1	2	40	0	2
Intestinal obstruction	9	1	11.1	1	0	3	33.3	3	0	5	55.6	0	5

hours before. The granules in it should have been Berg's granules. They did not stain so well with hematoxylin as the ones described in

the present material; they were coarser and less sharply outlined. Dr. Rich has shown me sections of the livers of rabbits which had received injections of foreign proteins. The similarity between the granules in the liver cells of these rabbits and those in the human cases was by no means a perfect one. Of course a comparison between the changes occurring in the tissues of animals and those occurring in man is not entirely satisfactory.

Berg (3) has observed the granules described by him in the liver of an executed man, and on the basis of his theory that they represent the storage of protein in the liver, one would expect to find them in the livers of all healthy people on a normal diet. I have failed to find the type of granule here described in the livers of seven apparently healthy people who had met death by violence.

Furthermore, whenever granules of the present type are found in the liver, they are usually present in the cells of the adrenal cortex as well. Berg's granules have never been described in the adrenal, and, if they represent protein storage in the liver cell, as Berg claims, they would have no obvious reason for existence there on the basis of our present information. In 61 of the 68 cases in which the liver was rated ++, the adrenal was well enough preserved for study. In 53 of these 61 cases the granules were there too.

Attention should be called to a brief study by H. E. Never (4), which deals perhaps with the same type of granules as that with which the present paper is concerned. Never set out to search for Berg's granules in pathological specimens and to study especially their relationship to iron storage and cloudy swelling. He is unaware of any previous demonstration of them in human material. He reports ten cases of cloudy swelling, in all of which he found pyroninophil granules, and seven cases without cloudy swelling, in none of which he found them. Most of his positive cases were from severe infections. His paper is the only one that I have been able to find in which granules similar to those of the present study are mentioned.

SUMMARY

The purpose of this paper is to call attention to a neglected pathological alteration in the cells of the liver and adrenal, and to point

out the relation of this change to the presence of infection. Patients who have died with severe infections very frequently show at autopsy conspicuous oval or elongated granules in the cytoplasm of the cells of the liver and adrenal cortex. These granules are well stained with hematoxylin and with pyronin. It must remain for future work to determine their precise nature.

This investigation has been aided in no small way by the numerous suggestions and constant encouragement of Dr. Arnold R. Rich. I want also to thank Dr. I. Gersh for his advice in the histological problems involved.

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THE CLINICAL AND PATHOLOGICAL FINDINGS IN CONGENITAL MALFORMATIONS OF THE HEART DUE TO DEFECTIVE DEVELOPMENT OF THE RIGHT VENTRICLE ASSOCIATED WITH TRICUSPID ATRESIA OR HYPOPLASIA

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True biloculate and triloculate hearts are extremely rare types of congenital malformations of the heart. The pseudo-biloculate and pseudo-triloculate hearts, *i.e.*, those caused by a defect in the development of one of the ventricles, with or without a defect in the development of the interauricular septum, are encountered with much greater frequency than the true biloculate and triloculate hearts. Among these cases the defective development of the left ventricle is rare, whereas a number of instances of a rudimentary right ventricle have been reported.

No attempt has yet been made to recognize this congenital malformation as a clinical entity, even though a number of cases of hypoplasia of the right ventricle have been recorded. In most instances only the briefest clinical history has been given. Cyanosis of varying intensity is always present. Blackford and Hoppe (1), in their report of a case of a pseudo-triloculate heart due to tricuspid atresia, stated that the electrocardiogram showed a left axis deviation. The roentgenogram of the chest which they showed is almost identical with those taken of our cases. Bellet and Stewart (2) also reported a case of atresia of the tricuspid orifice and a rudimentary right ventricle in which the electrocardiogram showed a left axis deviation. Kugel (3) in his report of a biloculate heart with a dextrocardia discussed the roentgenographic findings and commented on the irregularity of the upper right margin of the cardiac shadow and on the appearance of a single vessel at the base of the heart.

The following cases are reported because they illustrate that the failure in the development of the right ventricle presents a clinical syndrome sufficiently characteristic to enable the diagnosis to be made during life.

Case No. 1. J. H. (H. L. H. No. 94950). White male infant. First seen at the Harriet Lane Home in November, 1935, at the age of 4 months with the complaint of attacks of dyspnea and cyanosis.

Family History: Non-contributory.

Past History: The infant was a full-term baby weighing 7 pounds. There was marked cyanosis at birth. After the first week the infant's color improved and thereafter, until the day before admission, the mother had noticed the cyanosis only when the baby cried, although upon direct questioning she admitted that the infant's fingernails were "blue all the time."

The day before admission, the baby appeared short of breath and blue. He had a series of convulsions and for this reason was brought to the hospital.

Physical examination showed the following positive findings: The infant was intensely cyanotic and dyspneic. The *chest* showed no deformity. The *heart* was enlarged to the left. The apex could be felt in the anterior axillary line. There was no shock, no thrill and no murmur. The second sound was heard better over the pulmonic than over the aortic area. There was, however, no splitting of the second sound at the base. The *lungs* revealed a few râles. Although the *liver* was only slightly enlarged, a pulsation was detected at its margin. The *extremities* showed intense cyanosis, but no clubbing and no edema.

The *laboratory examination* showed: 6,300,000 red blood cells; 19,400 white blood cells. Hemoglobin, 118 per cent. The urine was negative. The Wassermann reaction of the blood was negative. The skin reaction to 0.1 mg. of tuberculin was negative.

Fluoroscopic examination showed that the enlargement of the heart was entirely on the left side and that the heart shadow extended outward almost at right angles to the sternum. There was a striking absence of the pulmonary conus and a narrow aortic shadow (Fig. 1). The right auricle could not be clearly visualized. When the baby was rotated into the left anterior oblique position, the right ventricle was strikingly small, and the great vessels appeared as a single trunk (Fig. 2). In order to visualize the relation of the left auricle to the esophagus, the infant was fed a dilute barium mixture and then examined under the fluoroscope in the right anterior oblique position. By this method the left auricle appeared to be of normal size.

The *electrocardiogram* showed a normal sinus mechanism, a rate of 150 per minute, a P-R interval of 0.12 second, and a left axis deviation (Fig. 3).

Clinical Impression: Biloculate heart due to the absence of the right ventricle and the right auricle.

The evidence of the absence of the right ventricle was seen both in the roent-

genogram and the electrocardiogram. The absence of the right auricle was incorrectly postulated; it was hidden behind the sternum.

One month after admission the infant died.

Autopsy (No. 14525), performed by Dr. Oppenheimer, showed a triloculate heart. The *right auricle* was tremendously dilated; the superior and inferior venae cavae entered in a normal fashion. There was complete atresia of the tricuspid valve; the only indication of the tricuspid valve was a slightly puckered, dense, gray scar. There was a slit-like opening, 2.5 mm. in length, in the interauricular wall through which a probe could be passed into the left auricle. This opening was 4 mm. above the attachment of the aortic leaflet of the mitral valve. It appeared as a perforation through the septum between the two auricles rather than a true foramen ovale, since it did not lie in the position of either of the two embryological openings. This was the only opening of any size through which the blood could leave the right auricle. There were, in addition, several tiny perforations in the interauricular septum. The *left auricle* was comparatively small; the pulmonary veins entered normally. The mitral valve was normal in appearance. The *left ventricle* was tremendously dilated and hypertrophied. There was no interventricular septum. The aortic valve was of normal width (3 cm.) and had three cusps. The right coronary artery was misplaced, and opened behind the third cusp instead of behind the posterior cusp. Just beyond the left subclavian artery was a large and anomalous patent *ductus arteriosus* leading to the pulmonary artery.

There was no trace of a *right ventricle*. The *pulmonary artery* proximal to the ductus arteriosus was small and atrophic. It ended blindly in a pouch lined with thickened intima. The only indication of the pulmonary cusps was the existence of a few little ridges and depressions.

Anatomical Diagnosis: Congenital malformation of the heart—cor triloculare; complete atresia of the tricuspid and pulmonary valves; complete absence of the right ventricle; anomalous patent ductus arteriosus; patent foramen ovale; hypertrophy of the right auricle and the left ventricle.

Case No. 2. B. G. (H. L. H. No. 94619). Colored female infant who was first brought to the Harriet Lane Home in December, 1935, at 5 weeks of age, because of "a swelling on each hip."

Family History: Both parents were known to have syphilis. The father had received no treatments and the mother only irregular treatments. She had had seven children and no miscarriages.

Past History: The infant was a full term baby weighing 8 pounds. The cord Wassermann was positive. There was cyanosis at birth which was reported to have improved considerably after the first ten days of life. The infant had persistent snuffles. At the age of 4 weeks the baby developed bruises on the outer aspect of both thighs for which she was brought to the hospital.

Physical examination showed the following positive findings: The infant was

a well nourished, well developed, colored baby with rapid, noisy respirations associated with a bloody nasal discharge and intense cyanosis.

On both *thighs* were swollen areas approximately 6 x 4 cm. in diameter; these were symmetrically placed, raised, and indurated. In the center of the lesion on the right thigh there was a punched-out ulcer 0.5 cm. in diameter which contained caseous material. The lesion on the left thigh was similar in size. The skin in the central area was denuded and showed a red, raw surface. There was a similar indurated area on the right leg which had not ulcerated. Biopsy revealed fat necrosis.

The *heart* was enlarged. The left border extended just outside the midclavicular line. There was no shock, no thrill and no murmur. The sounds were of good quality. There was no splitting of the second sound at the base. The *lungs* were clear. The *liver* was one fingerbreadth below the costal margin; there was, however, no pulsation at its margin. The *extremities* showed marked cyanosis, but no clubbing and no edema.

The *laboratory studies* showed: 7,500,000 red blood cells; 12,800 white blood cells. Hemoglobin, 152 per cent. The skin reaction to 1.0 mg. of tuberculin was negative. The blood Wassermann was negative. The roentgenogram of the extremities did not show pronounced periosteal changes but were suggestive of lues.

The *roentgenogram* of the heart showed a shadow identical with that of the first case (Figs. 4 and 5). The oblique view was not taken at precisely the same angle, which accounts for the difference between Figures 2 and 5. Under the fluoroscope the cardiac shadow appeared to be identical with that of the other infant. In both cases, the upper margin of the cardiac shadow was rectangular in shape and made a sharp angle with the sternum due to the absence of the shadow which is normally cast by the pulmonary conus of the right ventricle. Both showed a narrow aortic arch. In the left anterior oblique position the right ventricle in each instance appeared to be small.

The *electrocardiogram* was also strikingly similar to that of the previous case. It showed a normal sinus mechanism, a rate of 140 per minute, a P-R interval of 0.12 second, and a left axis deviation (Fig. 6).

Clinical Impression: Inasmuch as the only clinical difference between these two cases was the absence of pulsation at the margin of the liver, it was postulated that there was a similar congenital malformation of the heart with a widely patent foramen ovale permitting free flow of the blood from the right to the left auricle, *i.e.*, functionally a biloculate heart with tricuspid atresia, an absent right ventricle, a pulmonary atresia, and a patent ductus arteriosus.

The infant did well in the hospital. The lesions on the thighs healed slowly, and at the end of a month she was discharged to her home, to be followed in the dispensary.

On February 2, 1936, she was brought back to the hospital with a pneumococcus meningitis and died seventy-four hours later. At the time of her second admission, there was a loud systolic murmur heard all over the precordium.

It was loudest in the second left interspace but harshest in the fourth left interspace. Compensation was good. She died at the age of 3½ months of pneumococcus meningitis.

Autopsy (No. 14640), performed by Dr. Jarcho, showed the following positive findings: The *heart* was enlarged. It weighed 48 grams. The *right auricle* was enlarged. The superior and inferior venae cavae entered in a normal fashion. The *foramen ovale* was widely patent, measuring 2 cm. in diameter. The *tricuspid valve* was represented by a tiny white endocardial thickening, measuring 7 mm. in length; below this there were a few tiny strands suggestive of chordae tendineae. These converged on a tiny rudimentary muscle. The entire *right ventricle* was 1.5 cm. in diameter and 1 cm. in length. It was lined by thick white endocardium and completely filled with an old organized thrombus. There was complete atresia of the pulmonary orifice. The *pulmonary artery* was hypoplastic, proximal to the *ductus arteriosus* which was widely patent. The *pulmonary valves* were represented by three shallow crypts—each less than 2 mm. in diameter. The *left auricle* was normal. The pulmonary veins entered normally, and the mitral valve was normal. The *left ventricle* was hypertrophied. Its wall measured 1.1 cm. in thickness. The aortic valves were normal, and the coronary arteries opened in their usual positions. There was a shallow depression in the interventricular septum, but there was no demonstrable connection with the rudimentary right ventricle.

The *brain*, especially over the frontal lobes and the base, was covered with a thick, yellowish-green exudate. Pneumococci were cultured.

The *bones* showed no evidence of congenital lues.

Anatomical Diagnosis: Congenital malformation of the heart; hypoplasia of the right ventricle and the tricuspid valve; patent foramen ovale; atresia of the pulmonary artery; patent ductus arteriosus. Purulent meningitis (Pneumococcus Type IV).

DISCUSSION

The essential feature of the malformation in these two cases is the diminutive right ventricle. A non-functioning right ventricle is always associated with serious underdevelopment of the tricuspid valve, either tricuspid atresia or marked hypoplasia, and a severe malformation of the pulmonary tract, either a transposed pulmonary artery or pulmonary atresia. If there is pulmonary atresia, there must be a patent ductus arteriosus in order to have the circulation compatible with life.

The outstanding clinical manifestation is persistent cyanosis; murmurs, if present, are of secondary importance. The cyanosis is present at birth. Although the cyanosis may decrease in intensity

during the first few days of life, it does not entirely disappear, and during the ensuing weeks it increases steadily. The temporary decrease in cyanosis presumably is due either to the initial fall in the hemoglobin or to the slowness in the establishment of the pulmonary circulation through the abnormal channel.

As regards murmurs, the only abnormal opening in this malformation which could cause a murmur is the ductus arteriosus. The murmur produced by a ductus arteriosus depends primarily not upon the patency of the ductus but upon the relative pressure in the systemic and pulmonic circulations which are connected through the patent ductus. Whenever the pressure in the systemic circulation is higher than that in the pulmonic circulation, the blood is forced by the high pressure in the aorta through the ductus into the area of low pressure, thus producing a systolic murmur. If the difference between the diastolic pressures of the two circulations is sufficiently great, the blood continues to be forced through the ductus during diastole, causing the continuous murmur characteristic of a patent ductus arteriosus. On the other hand, whenever the pressure between the two circulations becomes approximately equal, even though the ductus remains patent, no murmurs are heard. Therefore, during this age period a murmur may or may not be present in cases in which the ductus arteriosus is patent.

As a matter of fact, auscultation is of diagnostic aid only in determining the quality of the second sound at the base. The significant characteristic of this sound is its purity. It is a strong clear sound without the slightest suggestion of splitting or reduplication. It is also noteworthy that, unless the aorta and the pulmonary artery occupy their normal positions, no reliance can be placed on the relative intensity of the second sound to the right or to the left of the sternum. The significance of this is illustrated by the fact that in the first case, even though there was complete atresia of the pulmonary valve, the second sound was heard better to the left of the sternum than to the right.

The diminutive size of the right ventricle can be recognized by means of the roentgenogram and the electrocardiogram. Indeed, it would appear that the shape of the heart in this malformation has quite a characteristic outline in the roentgenogram. The two

plates were almost identical (compare Figs. 1 and 4). The characteristic feature in the roentgenograms is the narrow aortic arch combined with the shape of the upper margin of the cardiac shadow to the left of the sternum. The curve of this shadow is concave instead of convex. Its normal convexity is due to the pulmonary conus of the right ventricle. In this malformation there is virtually no right ventricle; hence the upper margin of the cardiac shadow at the cardiosternal junction becomes concave.

A narrow shadow cast by the great vessels is not necessarily indicative of pulmonary atresia, since it is also seen in transposition of the great vessels. In these cases, the narrow aortic shadow was due to the absence of the pulmonary artery; whereas in cases of transposition it is due to the fact that the pulmonary artery lies behind the aorta. The two malformations are, however, readily differentiated by the size of the right ventricle. In the former the right ventricle is diminutive in size; in the latter it is greatly enlarged.

Fluoroscopic examination not infrequently gives a more accurate visualization of the aortic arch than is obtained from the roentgenogram because any slight rotation of the head changes the relation between the superior vena cava and the great vessels and thereby greatly alters the width of the aortic shadow. Furthermore, by rotating the infant into the left anterior oblique position evidence is obtained concerning the size of the right ventricle and also of the structure of the aortic arch. When the right ventricle is enlarged, it appears to approach the anterior chest wall. In contrast to this, in cases with a small right ventricle there is no appreciable cardiac shadow anterior to the aorta. This feature is striking, although the cardiac shadow in itself does not appear to be smaller than that of a normal heart owing to the large size of the left ventricle. The aorta, in cases of pulmonary atresia, is sharply outlined as a single great trunk due to the absence of the pulmonary artery; whereas, in transposition of the great vessels, the shadow cast by these vessels increases in width in this position because of the visualization of the pulmonary artery which lies behind the aorta in the A-P view.

The electrocardiogram is another aid in diagnosis. A diminutive

or absent right ventricle causes a left axis deviation in the electrocardiogram. This malformation is, so far as I know, the only malformation of the cyanotic group which is associated with a left axis deviation in the electrocardiogram.[1]

A markedly hypoplastic or absent right ventricle is, as previously mentioned, always combined with serious underdevelopment of the tricuspid and the pulmonary valves. Indeed, Dr. Maude Abbott believes that the two principal causes of hypoplasia of the right ventricle are (1) tricuspid atresia and (2) pulmonary atresia.[2] It makes no significant difference which of these is primary; in either case the failure of the right ventricle to function in turn affects the other valve so that the resulting malformation is one of marked hypoplasia of the tricuspid orifice, the right ventricle, and the pulmonary tract.

The fact that the right ventricle does not function means that the blood is unable to leave the right auricle through its normal channel. It must, therefore, escape by way of the left auricle. For this reason the existence of some defect or patency in the interauricular septum is inevitable.

The size of the opening in the interauricular septum determines whether the heart functions as a biloculate or a triloculate heart. If the interauricular septum is absent or there is a large patent foramen ovale, the blood flows freely from the right auricle into the left auricle, and the heart functions as a biloculate one. In contrast to this condition, if there is a well formed interauricular septum with only a small septal defect, *i.e.*, functionally a triloculate heart, the interauricular septum offers obstruction to the outflow of blood from the right auricle. The consequence is that the auricular pulsation is transmitted to the liver and causes the presystolic pulsation which is palpable at its margin. Thus, this congenital malformation gives the same clinical sign as that found in an adult with acquired tricuspid

[1] Through the kindness of Dr. Lawson Wilkins, I have had the opportunity to see the electrocardiogram of a similar case taken at ten days of age. During the first weeks of life a right axis deviation in the electrocardiogram is physiological. It is significant that in this case at ten days of age there was a balanced electrocardiogram.

[2] Personal communication.

stenosis. In the first case the presence of a pulsation at the margin of the liver was recognized during life, but the significance of this finding was not appreciated until after the autopsy has been performed.

The only other condition in which a pulsating liver is found is that of tricuspid insufficiency. Under such circumstances, the pulsation is systolic in time, and furthermore it is associated with a dilated right ventricle. In contrast to this, the presystolic pulsation of this functionally triloculate heart is associated with a diminutive or absent right ventricle. It follows that in this malformation the differentiation between a functionally triloculate and biloculate heart can be ascertained by the presence or absence of a pulsating liver.

SUMMARY

The central feature in this congenital malformation is the diminutive size of the right ventricle. The associated malformations can be postulated from the fact that the right ventricle does not function. The failure of the ventricle to function means that neither the tricuspid valve nor the pulmonary valve can function, and hence they are either atresic or markedly hypoplastic. Furthermore, it means that the only way for the blood to escape from the right auricle is through some defect in the interauricular septum. The extent of this defect determines whether the heart functions as a biloculate or triloculate heart.

Clinically, the malformation is associated with persistent cyanosis and no murmurs. The diagnosis is established through the recognition of the diminutive size of the right ventricle. This condition can be recognized in the roentgenogram by the peculiar outline of the cardiac shadow. In the A-P view, because of the absence of the pulmonary conus, the upper contour of the cardiac shadow immediately to the left of the sternum has a concave instead of a convex outline. In the left anterior oblique position the small size of the right ventricle is indicated by the absence of cardiac shadow anterior to that of the aorta. These observations are substantiated by the electrocardiogram which shows a left axis deviation.

The differentiation between a functionally biloculate and a triloculate heart depends upon the size of the interauricular septal defect.

If there is free communication between the two auricles, *i.e.*, functionally a biloculate heart, physical examination yields no additional positive findings. In contrast, when the interauricular septum is well formed, it causes obstruction to the outflow of blood from the right auricle. Under these circumstances, the auricular pulsation is transmitted to the liver and is readily palpable at its margin. It is this pulsation at the edge of the liver occurring in conjunction with a diminutive right ventricle which distinguishes a functionally triloculate from a functionally biloculate heart.

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PLATE 1

Fig. 1. Roentgenogram of the Chest of Case 1 (J. H.) Taken in the A-P Position

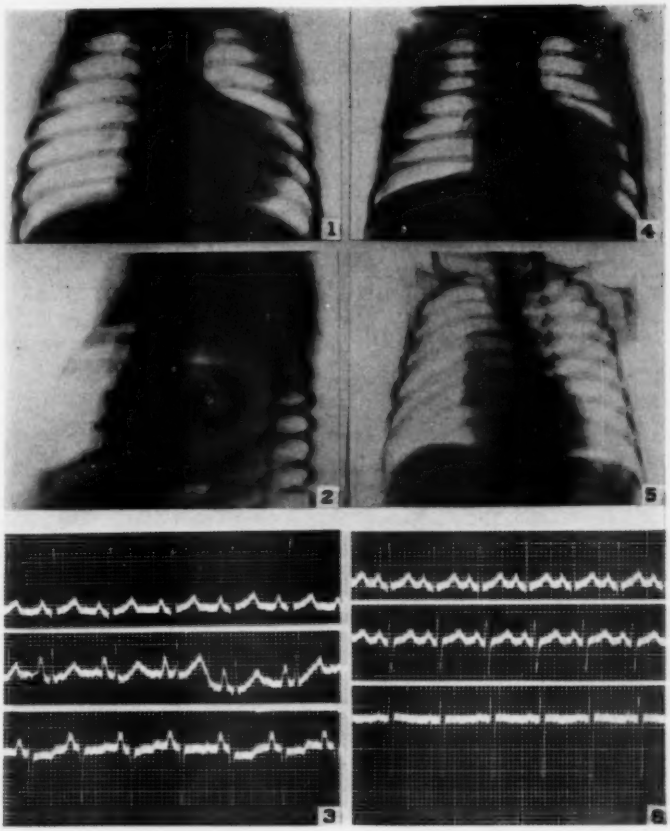
Fig. 2. Roentgenogram of the Chest of the Same Infant Taken in the Left Anterior Oblique Position

Fig. 3. Electrocardiogram from Case 1 (J. H.)

Fig. 4. Roentgenogram of the Chest of Case 2 (B. G.) Taken in the A-P Position

Fig. 5. Roentgenogram of the Chest of the Same Infant Taken in the Left Anterior Oblique Position

Fig. 6. Electrocardiogram from Case 2 (B. G.)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A NOTE ON THE NAIL-FOLD CAPILLARIES IN NEGROES[1]

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In the course of some observations upon capillary blood flow in the skin at the base of the finger nail we have had occasion to examine a number of negro subjects. The method which we have used is that which is in common use for such studies: a drop of oil is placed upon the skin at the base of the nail and the area is examined under a high-power binocular microscope, oblique illumination being used. We had previously imagined that negroes were not suitable subjects for such studies. In fact, one can find in the recent literature statements to the effect that "In negroes this method is impossible because of the pigment" (1). It has been our experience that in most negroes the nail-fold capillaries are clearly visible and quite as suitable for study as the capillaries of white subjects. We have examined negroes of both sexes, and of various ages and shades of color, and in only a single instance has the cutaneous pigment prevented visualization of the capillary circulation. In subjects of the lighter shades of color no difficulty has been encountered, but in several very dark subjects there have been small areas in the nail-bed where the view of the capillaries has been obscured by pigment. These heavily pigmented areas, however, are generally separated by grossly paler areas, about one millimeter in diameter, in which the superficial cutaneous pigment is lacking. Through the minute windows thus formed in the pigment-layer the capillaries are frequently more clearly visible than they are in the average white subject. In the one unsatisfactory subject mentioned above no such windows were present and only the hazy outlines of the capillaries could be seen through a screen made up of a continuous sheet of granular brown pigment. Whether the dis-

[1] Aided by a grant from the Helen Hartley Jenkins Fund.

[2] Jacques Loeb Fellow in Medicine.

continuity of the cutaneous pigmentation of the nail-fold which we have encountered in most of our subjects would also be found in negroes of pure African stock we have been unable to ascertain, for we have examined no such individuals. We have made no attempt to study the constitutional differences between the capillaries of negro and white subjects (2), but our attention has been attracted by the fact that in many negroes, at comfortably warm room temperatures, the capillary blood flow appears more sluggish than in most white people.

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BOOK REVIEWS

Physical Therapy for Nurses. By RICHARD KOVÁCS. 286 pp. illus. $2.75. (*Lea & Febiger, Philadelphia, 1936.*)

This book will appeal to the physiotherapist because it is small. The little there is to say on the subject it says briefly and clearly. Perhaps it would have been just as well to have omitted the chapter on elementary physics; though excellent in itself, it is not needed in a book of this kind.

What one cannot help liking about this little volume is the absence of "Amateurishness, pretending to be professional," so disturbing in most books in the parts that attempt to set forth the "Technique" of the numerous "Therapies" grouped under the inclusive term "Physical Therapy." It occurs to this reviewer, that this is the first work to include in one treatise all of the subjects.

There is a feeling that too much emphasis is put upon the virtues of "Diathermy." Giving an old thing a new name was a commercial trick, a dangerous and misleading procedure. The author's admission that a large array of apparatus is not at all necessary, calls for more than mere commendation. Some means of applying heat and cold, together with ability to make deft use of his or her hands, is sufficient to accomplish wonders. To quote from page 13: "There is danger of too much and too complicated apparatus for physical therapy, just as there is danger in too much apparatus for diagnostic purposes, when one's five senses and clinical experience unaided should be adequate to solve many problems."

Apparatus is merely labor saving. Knowing how to operate it, how to turn on and off switches and valves does not constitute physical therapy. Just as in the rendition of a musical composition, the instrument and knowledge are but convenient tools, so, in attempting to make use of physical means in the treatment of the sick and injured, deep sympathetic feeling, a sense of rhythm and harmony, and an eye that sees into the soul of the sufferer, are necessary to round out the training of the physiotherapist.

W. H. R.

Medical Mycology. By CARROLL WILLIAM DODGE. 900 pp. illus. $10.00. (*C. V. Mosby Co., St. Louis, 1935.*)

Although the sub-title of this book is 'Fungous Diseases of Men and Other Mammals,' it might more aptly be 'Pathogenic Fungi of Men and Other Mammals' since relatively little space is devoted to the description of fungous diseases. The first five chapters deal with morphology, physiology and methods for the study of fungi. The remaining seventeen chapters are concerned predominantly with systematic medical mycology. It is a reference book rather than a text book, and as such should be very useful. Although there is great difference of opinion as to the classification of pathogenic fungi, so that no work on this subject can be

regarded as authoritative, the author gives full discussion of the opinions of other systematists and furnishes a very complete bibliography. The book is well illustrated, well arranged and well indexed. Among publications in the English language it is much needed.

J. H. B.

Convalescent Care in Great Britain. By ELISABETH GREENE GARDINER. 163 pp. $1.50. (*The University of Chicago Press, Chicago, 1935.*)

Great Britain is far ahead of the United States in her provision of convalescent care, having 431 institutions with approximately 24,000 beds as compared with 179 institutions with 8747 beds in the United States. This comparison is even more striking when it is found that Great Britain has 53.6 beds per hundred thousand of population as compared with 7.1 beds for the same number of people in the United States. Since a large proportion of the institutions for convalescent care in the United States are in the metropolitan district of New York City, the others being widely scattered throughout 24 States, it may be said that convalescent care, so long a recognized medical need in Great Britain, is almost non-existent with us.

The study on which this book is based was made in 1931, when the author was attached to the University of Liverpool, through an exchange lectureship, and had the opportunity of visiting many representative institutions for convalescent care and conferring with British leaders in this field. Mrs. Gardiner's book should serve a very useful purpose in directing attention in this country to this neglected field, as well as giving us the advantages of British experience in the care of convalescents.

The development and organization, the types and standards of care, legal supervision and regulation, and the financial aspects are discussed. The history of convalescent care in Great Britain is an interesting commentary on social evolution, and indicates the various social forces, such as bad housing, over-crowded hospitals and, finally, the preventive concepts of medicine that have joined to stimulate the development of the many British institutions.

The development of medical care in the United States has naturally proceeded along lines somewhat different from those adopted by Great Britain, and our out-patient departments have perhaps played a larger rôle in the after-care of hospital patients. There is, however, a great need in this country for institutions for the rehabilitation of patients after serious illness, so that they can take up again the routine life of industry and of household duties, and thus prevent incipient physical and emotional disorders from progressing to burdensome invalidism. Convalescent care has a definite place as an important unit of a complete health service in the United States.

The costs of adequate medical care are lowered by well-organized facilities for convalescent care, and for this reason, as well as for their high humanitarian value, the development of institutions for the care of convalescents should be

given serious consideration by people in all parts of this country. The British hospitals for convalescents have been maintained unimpaired and have even been extended during the ten-year period of financial depression in Great Britain. This is an indication of the value placed upon them by the public in that country.

Printed in mimeograph form, this book is a Social Service Monograph, edited by the Faculty of the University of Chicago. The author is Assistant Professor and Supervisor of Medical Social Work in the University of Minnesota.

G. C. R.

Theory and Practice of Psychiatry. By WILLIAM S. SADLER, C.V. $10.00. (*Mosby Company, St. Louis, 1936.*)

This book of 1231 pages is designed by the author as a "Psychiatric Textbook for Neuropsychiatric Specialists and General Practitioners of Medicine," and as "A Reference Handbook for Psychologists, Sociologists, Pastors, and Other Professional Readers." The writer expresses the hope in the preface that the work "will assist the medical profession in divesting mental hygiene of many of its psychiatric mysteries and deliver it from the 'confusion of tongues'—the sectarian clamorings—that have served so to obscure the 'common-sense' methods of study and practice which the rank and file of the profession are well qualified to undertake, if they are once delivered from the 'mystifications' of the multifarious teachings of the conflicting specialized schools of psychiatry." The book attempts to cover in scope, theory and treatment from ancient psychiatry dating back to 4000 B.C. to the last word of modern times.

Part I deals with "Theory of Psychiatry," comprising 12 chapters; part II deals with "Personality Problems," in 13 chapters; part III with "The Neuroses," in 22 chapters; part IV with "The Psychoses," in 7 chapters; part V with "Psychotherapeutics," in 19 chapters. Under "Psychotherapeutics," which is especially designed for the audience to whom the book is written, we have a wide range of topics covering every field of departmental knowledge, with such titles as "The General Management of Mental and Nervous Disorders," "The Physician As a Personal Counselor," "Suggestion," "Hypnotism," "Remotivation," "Sublimation and Substitution," "Habit and Self-Control," "Will Power and Decision," "Socialization," "Religious Therapy," "Philosophies of Life," "The Therapeutic Study Program," "Occupational Therapy" and 12 pages entitled "Physical Treatment." As would naturally be supposed from the scope of the production, its value is more encyclopedic than academic or therapeutic. The writer does ample justice to the views of the various schools of psychiatry and psychology, and is fair in his estimates of the assets and liabilities of these various points of view. There is, however, a strong trend of didactic emphasis throughout the whole volume which expresses itself in a reiteration of the word "technic." For example, in chapter 5 on "The Management of Fear" there is a subdivision entitled "Technic of Mastering Fear," with eight headings as follows: "Do not fear it," "Do not fight it," "Do not disown it," "Do not try to run away from it," "Utilize fear as a fight-stimulus," "Refuse to foster fear curiosity," "Unmask fear," "Avoid the

fear habit." These recipes sound logical, but when applied to individuals with actual fear problems that range all the way from nervous fears of neuropathic constitutions to the panic states of the psychotic and pre-psychotic, they would be very difficult and in fact dangerous for the psychologist, the sociologist, the pastor and the professional readers to apply to individuals seeking help and guidance. Chapter 10, which deals with the examination of a patient, is very comprehensive, and should give the practitioner an excellent idea of the size of his job in approaching and dealing with "nerves" with which he is so apt to be cursory.

One wishes that the writer had given us more case studies illustrative of theoretical material, drawing on his rich experience as an excellent and successful practitioner. The book is a good library volume for reference and classification concerning disputatious points of psychiatric and psychological therapy with their wealth of terminology from which nothing seems to have been omitted that is capable of a definition. The psychiatric practitioner will welcome this book as an addition to his library to which he can turn quickly for encyclopedic reference; the general practitioner will feel that he gets a liberal orientation in the field of neuropsychiatry. But while the general practitioner and the audience of lay readers will undoubtedly find themselves more conversant with the field of the behavioristic sciences by reason of a clear and concise presentation of material in this field, one can not help wondering whether or not they will find themselves better able to deal with concrete human problems after finishing this volume.

E. L. R.

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